HETEROTROPHIC BACTERIAL ACTIVITY IN SELECTED AQUACULTURE SYSTEMS NEAR COCHIN

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JUNE 1992

DEDICATED TO MY FATHER

AND IN LOVING MEMORY

OF MY MOTHER

DECLARATION

I hereby declare that this thesis entitled **"HETEROTROPHIC BACTERIAL ACTIVITY IN SELECTED AQUACULTURE SYSTEMS NEAR COCHIN"** is a record of original and bonafide research carried out by me under the supervision and guidance of Dr. V. Chandrika, Scientist, Central Marine Fisheries Research Institute, Cochin and that no part there of has been presented before for any other degree in any University.

Cochin-682 031,

June, 1992.

S. Santhi Thirumani

(S. SANTHI THIRUMANI)

CERTIFICATE

This is to certify that the thesis entitled **"HETEROTROPHIC BACTERIAL ACTIVITY IN SELECTED AQUACULTURE SYSTEMS NEAR COCHIN"** embodies the research of original work conducted by **Miss. S. SANTHI THIRUMANI** under my supervision and guidance. I further certify that no part of this thesis has previously formed the basis of the award of any degree, diploma, associateship, fellowship or other similar titles or recognition.

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PREFACE

The interplay between production, consumption and decomposition are the three important eco-regulatory and balancing process in aquaculture ponds which determine and regulate the output levels. Various microbial communities are involved in each of these ecological processes and their activities constitute the basis of trophic interactions. By regeneration and transformation of nutrients bacteria form the first link connecting the live organisms with the abiotic factors. However, the pond fertilisation practices have been so far based largely on the chemical factors and plankton levels. Only in recent years microbiological investigations with reference to aquaculture productivity and heterotrophic activity have been initiated, and the present work gives an account on the influence of hydrological parameters on heterotrophic activity in seasonal and perennial ponds of Narakkal, Cochin.

The measurement of heterotrophic activity of bacteria in aquatic environment had started as early as 1962. This is defined as the process by which the carbon autotrophically fixed into organic compounds by photosynthesis is transformed and respired by many heterotrophic microbes. The present work deals with aerobic microbes in the aquaculture pond and the work is restricted to surface and bottom water microbial heterotrophy only, eventhough it is well recognised that a great deal occurs in sediments.

The pathways of carbon and energy flow in aquaculture pond foodwebs can only be answered with a production measure, for example, if

microbes are being fed upon by protozoans and higher filter feeders, then the production rate of microbes is an integral part of the calculation of energy flow. There is still no single commonly agreeable method for measuring bacterial growth as respiration and so the literature is replete with arguments about different methods. Many of the arguments would have been ended if a single method was available, no matter how laborius or difficult, as a standard for calibration of other easier methods. The techniques for measuring production have been developed for a number of groups of marine organisms. But the problem for heterotrophic microbes, is that they live in a dilute environment where every process is difficult to measure. This is further confounded by the fact that microbes respond rapidly to any change in their surroundings so all incubations may influence results. Some practical problems with heterotrophic measurements include that it cannot be measured by adding a single radioactive - labelled compound (eg. glucose) and measure the rate of its use by microbes. This contrasts with algal primary production which may be estimated by following the metabolism of a single compound such as oxygen and carbon-dioxide.

Microbes in the aquaculture ponds are able to increase and decrease activity over wider ranges than any other group of organisms. If environmental conditions are favourable they grow rapidly. So the mere demonstration of the presence of 1×10^6 cells per ml in the environment does not reveal anything immediately about their production rate. Higher organisms either respire continuously or must enter distinctive resting states. For this reason, we learn a great deal more from their presence when we try to interpret energy flow.

Microbial death and grazing by protozoans occur at the same time as production but their importance in the aquaculture pond is unknown. As dissolved organic matter from solution is used mostly by flagellate and mussels it makes it difficult to infer microbial production from mass balance. Techniques with short incubations are the only way for estimating bacterial production.

The points given above stress the difficulties of studying marine heterotrophy. We now have a number of promising techniques for measuring rates of aquatic heterotrophic activity and microbial growth in the environment like measurement of a change in concentration of oxygen, carbon-dioxide or inorganic nutrients. Oxygen technique and the total carbondioxide measurements are used in the study of overall microbial activity. In situ studies depend both on analytical sensitivity and on the ability to describe mixing and gas exchange in the environment. The first useful isotope method is the ideal method for measuring the uptake and respiration of single organic compounds such as glucose, acetate or amino acids. This approach gives a relative measure of activity and information on conversion efficiency of these compounds but cannot be expected to give a measure of overall microbial metabolism.

Control of heterotrophy and of heterotrophic organisms is the area of interest as bacterial heterotrophy is a type of primary production in aquaculture pond since it fixes carbon which would otherwise have been lost to higher trophic levels. In ponds there is indirect evidence for light control over the standing stock of organisms and substrates.

The principal interest of the present investigation was to determine seasonal and vertical variation of chemoorganotrophic utilisation of glucose and sodium-acetate by the natural bacterial population in the aquaculture pond of Narakkal, Cochin using techniques which allow maintenance of the in situ gaseous concentrations during incubation. In addition salinity, dissolved oxygen, temperature, hydrogen-ion-concentration, primary production, plant pigments and total bacterial concentration were determined seasonally and vertically because of their possible relationship to chemoorganotrophy.

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I. INTRODUCTION

The present study "HETEROTROPHIC BACTERIAL ACTIVITY IN SELECTED AQUACULTURE SYSTEMS NEAR COCHIN" is concentrated on the kinetic parameters of natural microbial populations which has been used to study their activity in aquatic environment. Bacteria are the prime agents of the return of dead organic matter and known to play a major part in pond ecosystem. The quantitative and qualitative role of bacteria in the maintenance of the environment is the factor relatively unknown in the ecology of aquaculture ponds. Bacteria influence the pH and Eh, colour, carbonate content, oxygen tension, production of vitamins and other organic compounds of water and sediment and stands at the base of the fertility of the ponds.

Traditional methods of enumerating marine bacteria were based on plate counts, serial dilutions or phase contrast microscopy which gave estimates of at best 10% actual numbers and have generally been discarded for estimating bacterial biomass. Recently several chemical techniques have been used to estimate biomass. These include ATP (Karl and Holm-Hansen, 1977; Sorokin and Lyntsrev, 1978) muramic acid (Fazio et al., 1977; Moriarty, 1977) and lipo-poly saccharides (LPS) (Watson et al., 1977). However, for estimating the biomass natural assemblages in different physiological states these differ from the drawback of having varying conversion factors between cell component measured and bacterial biomass. The technique of epifluorescence microscopy has recently come into use for directly counting bacteria.

The progress in microbiology in the recent past is inextricably linked to the development of several new methodologies and instrumentation. Among these, the most significant has been the application of radio-isotopes as tracers. Apart from harnessing of radiotracers for studying metabolic reactions and pathways, radio-active compounds have also been used for studying turnover of molecules. The determination of actual in situ rates of utilization of DOC estimation of bacterial heterotrophy and bacterial primary productivity also became possible with the introduction of carbon-14 radio active tracer.

The most widely used techniques for the assessment of the microbial activity are based on the studies of Parsons and Strickland (1962) which was further improved by Wright and Hobbie (1966). The two methods are commonly employed for accomplishing a measurement of bacterial activity, the actual rate at which bacteria are using a given compound, the turnover of given compound in a system are the tracer approach (Williams and Askew, 1968; Azam and Holm - Hansen, 1973) and the Kinetic approach (Parson and Strickland, 1962; Wright and Hobbie, 1968).

The tracer approach involves adding labelled substrates at concentrations well below those known to be in their natural environment and using the measured fraction of available labelled substrate taken up to calculate turn over rate or turn over time (Wright and Burnison, 1979) the kinetic approach assumes that the responses of a natural microbial population to a range of concentration of added substrate can be used to describe natural heterotrophic activity. Strickland and Parsons (1972)

developed a quantitative radio-chemical method for measuring "heterotrophic potential" in seawater. They showed that labelled glucose and acetate were accumulated by marine plankton at a rate which correspond to Michaelis-Menten enzyme Kinetics. Wright and Hobbie (1966), Hobbie and Crawford (1969), Azam and Holm-Hansen (1973) have shown that heterotrophic bacteria do utilise dissolved substrates in microgram and even nannogram/litre concentrations. Glucose and acetate utilization by the natural microbial community in a stratified reservoir was studied by Donald Edward Francisco (1970). Gocke (1977) compared the turn-over of a variety of solutes with the two methods and obtained similar turnover times from estuarine and coastal waters. However Oligotrophic waters consistantly yielded shorter turnover times with the tracer techniques, and he attributed the discrepancy to the limitations of the kinetic approach where microbial activity is low and the kinetics often diverge from the expectations of Michaelis - Menten scheme, a problem explored in theory by Williams (1973). However, the two methods have been successfully employed to generate valuable new information on the natural activity of the heterotrophic bacteria.

A significant correlation between microbial population and V_{max} was found in coral atolls of Lakshadweep archipelago using C-14-labelled glucose, sodium acetate and glutamic acid by Chandramohan and Ramaiah (1987). Choquest et al. (1988) found out statistical considerations associated with the use of the heterotrophic activity method for estimating V_{max} for aquatic environment and very recently Moriarty et al. (1985) published

a comprehensive review on the productivity and trophic role of bacteria on coral reefs.

A planned programme of research for seasonal variation of microbial kinetic parameters and other ecological parameters were conducted during the three seasons viz. pre-monsoon, monsoon and post-monsoon periods of 1989 and 1990.

Two different prawn culture ecosystems were selected for the present study viz., a perennial prawn culture pond of Central Institute of Brackishwater Aquaculture (area 0.6 ha) and a seasonal culture pond locally called pokkali field (area 0.4 ha) all of which are situated at Narakkal, near Cochin.

The thesis is presented in VI Chapters, Chapter I - INTRODUCTION to the topic of study, a REVIEW of the status of radiotracer study in various marine environments of the world is included in order to bring an awareness of the present status of our knowledge in the subject and also stress the importance of such study to enhance the productivity of aquaculture operations in the coastal area to reduce the costly inputs of fertilizers.

Chapter II is on MATERIAL AND METHODS adopted for sample collection and experimental radio tracer techniques followed to study heterotrophic bacterial activity seasonally and vertically. In addition, regular samples of water and sediments were collected for hydrological parameters, primary production, plant pigments and total aerobic heterotrophs from two culture ponds (perennial and seasonal) monthly as well as seasonally.

Chapter III RESULTS of this investigation were presented under four parts. Results of glucose uptake experiments and acetate uptake experiments for kinetic parameters in surface and bottom water of the two aquaculture systems are in part 1 (a) and part 1 (b). The results of seasonal variations of hydrological parameters and sediment parameters are given in part-2 (a) and part 2 (b). Part-3 (a), the results of primary productivity (gross and net production), plant pigments (chlorophyll, a,b,c and carotenoids) and their ratio, and Part-4 the numerical variation of bacterial cells (TPC) in water and sediment samples during 1989 and 1990 were presented and explained.

Results of computer analysis of one way ANOVA were used to test the significance to effect of seasons and space on various parameters. The results of the experiments and statistical interpretation of the findings form the subject matter of Chapter III. All data collected on the above aspects are given either in the form of graphic intensity charts or tables for effective presentation of the results.

In Chapter IV, DISCUSSION and overall assessment of the inter-relations between abiotic environmental factors and heterotrophic activity of bacteria have been attempted. A critical evaluation of the implication of environmental factors influencing heterotrophic activity which in turn enhance the fertility of the pond during farming and to reduce the costly manuring inputs for economic production are discussed in detail.

An executive summary (Chapter V) of the results of investigation is presented in the final section of the thesis which is followed by a detailed list of references (Chapter VI) on the subject.

Historical Perspective

The beginnings of tracer methods date back to early 1920s when Hevesy and his co-workers used naturally occurring Isotopes. When artificial radio-activity was discovered by Joliot and Curie in early 1930s, Hevesy was also the first to study the distribution of the radio-actively labelled phosphate in animals. This experiment can be considered as the first use of radio isotopes as tracers to follow the course of a metabolite in living organisms and phosphate movements and reactions of a minutely small administered dose of labelled sodium phosphate were followed.

The radioisotope of carbon, ^{14}C, was first detected and made by Ruben and Kamen in 1940s. Later, processes for the manufacture of ^{14}C by the atomic reactor were derived from the pioneering discoveries of these investigators. From the time radio isotopes became available, experimental biology especially biochemistry made meteoric progress contributing on the way to the inception of a wholly new branch-molecular biology.

Some important isotopes used in biological research are ^{3}H (half life 12.34Y) ^{14}C (half-life 5570Y) ^{32}p (half-life 14.3d) ^{35}S (half-life 87d) ^{131}I (half-life 8d) and ^{125}I (half-life 60d). These isotopes are generally used for : (i) Gross distribution studies., drug distribution organ/cell distribution (ii) Metabolic pathways; biosynthesis, breakdown regulation, (iii) DNA duplication, transfer of genetic information, protein synthesis and (iv) rates of reactions in living organisms, precursor product relationships and pulse-chase experiments.

The importance of radiotracer techniques in chemistry and microbiology is evident from the information compiled in table 1.

Table 1. A comparison of total number of studies performed with labelled atoms during the last four decades

Journal	Year	% research papers having used radio-isotopes
J. Biol. Chem.	1945	1
	1955	39
	1965	59
	1988	99.5
Biochem. J.	1945	0
	1956	18
	1965	60
	1988	96

Marine Microbial tracer studies remained small with a long doubling time for decades only to undergo a pleomorphic change into a large rapidly growing field. In recent years there has been a marked shift away from consideration of pure culture studies in ecology. Early bacteriologists were forced to study the activities of mixed bacterial population because of the difficulties of obtaining pure culture. Koch simplified the pure culture technique and paid emphasis on microbial investigations to the study of pure cultures. This approach was the 'modus operandi' for all

microbiologists and the emphasis continued in the marine field until the mid 1960's.

In 1962, Strickland and Parsons proposed a technique to measure directly the 'in situ' metabolic activity of marine micro heterotrophs. This entailed adding a radio-active substrate directly to a water sample and measuring the amount of label incorporated into the particulate material after incubation. In principle, the technique was analogus to the method of Steeman Nielsen (1952) who developed the use of ^{14}C-$NaHCO_3$ to measure primary production in oceanic water. Wright and Hobbie (1965, 1966) investigated many aspects of this "heterotrophic potential technique" and expanded the theoretical basis for its application. Their efforts stimulated the wide-scale application of the technique. It has proven to be a powerful tool for examination of the in situ activity of microheterotrophs.

Saunders (1958) was the first to apply C-14 tracers of organic compounds to the study of in situ heterotrophy. He showed by a differential filtration technique as well as autoradiography that heterotrophic uptake by algae and bacteria could be measured and that it was principally due to bacteria. Oscillatoria tenuis and Anabaena flos-aquae were also capable of considerable heterotrophy. Goldman, et al. (1967) also showed heterotrophic uptake of ^{14}C-glucose. They observed that the uptake was greater in the light than the dark. Sorokin (1964, 1965) and other Russian workers (1968) measured the microbial utilization of organic carbon

as a constant proportion of dark $^{14}CO_2$-uptake. This concept has not been corroborated.

Parsons and Strickland (1962) developed a quantitative radiochemical method for measuring "heterotrophic potential" in sea water. They showed that labelled glucose and acetate were accumulated by marine plankton at a rate which corresponded to Michaelis-Menten enzyme kinetics (see Mahler and Cordes for a discussion of biochemical kinetics). The formula they proposed for the velocity of uptake is as follows (notation changed for convenience):

$$v = (cf\ (S_n + A)\ /C\mu t \qquad (1)$$

Where v is the velocity of uptake (µg/1/hr), c is the radio-activity of the filtered organisms (CPM), f is the isotopic discrimination factor, S_n is the natural substrate concentration (µg/1), A is the concentration (µg/1) of added substrate, C is the activity (CPM) of 1.0 µCi of the substrate in the particular counter used, µ is the number of µCi added, and t is the incubation time (hr). The f factor has been neglected for most subsequent work since its value is unknown, thought to be approximately unity. They developed an equation similar to the Lineweaver-Burk equation which could be used to determine the value of $(K_t + S_n)$ where K_t is an uptake constant analogous to the Michaelis constant, after determining the relative uptake at various concentrations of added substrate. S_n, in general, could not be measured in natural waters because of the limits of analytical methods. The values of $(K_t + S_n)$, however, were consistently less than 20 µgC/1. They, therefore, reasoned that by routinely adding

250 μgC/1 any effect of S_n in equation (1) could be neglected, and this equation could be used to make comparisons of the heterotrophic potential of diverse waters.

Wright and Hobbie (1965, 1966) and Hobbie and Wright (1965) found that the uptake of glucose and acetate by natural populations from Lake Erkin followed Michaelis-Menten Kinetics only at low concentrations. Uptake in the 0.5 to 5 mg substrate/l range followed first order kinetics. Therefore, they questioned the validity of the relative heterotrophic potential measurements of Parsons and Strickland (1962). They also developed an equation for describing substrate utilization which is based on a modification of the Lineweaver-Burk equation.

$$S/v = K_t/V_{max} + S/V_{max} \qquad (2)$$

Where S is the substrate concentration and V_{max} is the maximum velocity of uptake. In natural waters S_n is unknown and using the concept of Parsons and Strickland (1962) the S of equation (2) becomes $(S_n + A)$ as follows:

$$(S_n + A)/v = K_t/V_{max} + (S_n + A)/V_{max} \qquad (3)$$

Equation (1) is rearranged so that:

$$(S_n + A)/v = (C\,\mu t)/c$$

Substituting equation (4) into equation (3) and rearranging gives:

$$(C\,\mu t)/c = (K_t + S_n)/V_{max} + A/V_{max} \qquad (5)$$

Using this equation the uptake measurements at several low concentrations of added substrate were plotted with $C\,\mu t/c$ on the ordinate and A on

the abscissa (Figure 1). Thus, the slope is $1/V_{max}$ and the intercept on the abscissa is $-(K_t + S_n)$. The intercept on the ordinate is S_n/v which equals the turnover time, T_t, assuming constant production and removal of the substrate.

The experimental method developed by Wright and Hobbie (1965, 1966) and Hobbie and Wright (1965) consists of adding a series of different concentrations of radioactive substrates to subsamples of natural water. These are incubated in the dark at the in situ temperature. After an appropriate incubation period (determined by the rate of uptake so that no more than 5% of the radioactivity in any sample is removed), the plankton are filtered on to membrane filters and their radioactivity determined. This technique has been used with success in both fresh water and seawater. Vaccaro and Jannasch (1967) and Vaccaro (1969) have experienced some difficulty with the technique in ocean environments. Their data resulted in sigmoid functions instead of the linear relationship of the modified Lineweaver-Burk plot. This uptake may be due to kinetics not described by the Michaelis-Menten relationship or may be a manifestation of the community reaction to the added substrate.

Wright and Hobbie (1966) observed that this method assumes that the radioactive substrate taken up by the population remains in the cells during the experiment and is not immediately respired as $^{14}CO_2$. It also assumes that substrate is not excreted as ^{14}C-metabolic products. Hamilton and Austin (1967) showed that the amount of ^{14}C-glucose respired by a marine pseudomonad was quite significant under conditions roughly

approximating the Wright and Hobbie method. Their conclusions were that the rigorous use of the method to determine all the ecological and kinetic parameters theoretically possible was too arduous to have any application in the field of ecological investigations.

Hobbie and Wright (1965) and Wright and Hobbie (1966) have suggested the uptake method could be used as a bioassay for the natural substrate concentration. In the first, a bioassay organism which has a low K_t for the substrate in question is isolated. K_t for this organism is determined in pure culture experiments involving defined media. After K_t has been determined, the organism may be used as a bioassay organism by conducting the uptake experiment in filtered natural water. K_t is then substracted from $(K_t + S_n)$ derived from the experiment to yield S_n. The other method they suggested was to perform the uptake experiment as usual, but to parallel this, with an uptake analysis in which the sample has been diluted with water which does not contain the substrate. If the dilution were 1:1, the above would yield values for $(K_t + S_n)$ and $(K_t + S_n/2)$. These may then be solved simultaneously for S_n. These methods have been used with only limited success (Allen, 1968; Vaccaro et al., 1968; Wright and Hobbie, 1966).

Vaccaro and Jannasch (1966) suggested that the determination of K_t and the maintenance of a pure culture with a defined K_t was extremely difficult. Furthermore, they were dubious of obtaining suitable dilution water for the second method. They were, however, able to obtain several reliable values in seawater for S_n of glucose. These were con-

siderably higher than values obtained in fresh water by Wright and Hobbie (1966) and Allen (1968). Vaccaro, et al. (1968) compared results of the chemical (Hicks and Carney 1968) and bioassay procedures for glucose concentration in seawater and found concentrations which varied from considerably more to considerably less than had been determined previously.

Vaccaro and Jannasch (1967) found that ageing or enriching seawater often allowed an interpretable uptake analysis when unaged or unenriched water did not. Vaccaro (1969) investigated in detail the response to enrichment in several sea water samples. His results suggested that earlier difficulties in obtaining linear response was due to virtually no uptake by a presumably small population. Enrichment increased the number of organisms capable of utilizing the substrate and Michaelis-Menten uptake Kinetics resulted. He also showed that enrichment was a possible source of a population with a defined K_t.

Harvey (1955) Rittenburg (1963) and Wood (1967) have stressed the uncertainity and shortcoming in our knowledge of the qualitative aspect of microbial activity in the sea. The principal role of the heterotrophic microorganisms is in the biology of the respiration of organic compounds and the consequent regeneration of the inorganic nutrients. Calculations dependent upon the observed short-term uptake of labelled ^{14}C from glucose and acetate by the natural population were used to estimate both the natural concentration of specific organic substrates and some relative rates of cellular incorporations. More elaborate procedures were later used by Wright and Hobbie (1965) and Hobbie and Wright (1965) in their

work on lakes. While generally endorsing the theory and application advanced by Parson and Strickland these authors also recommended a bio-assay procedures for estimating natural substrate concentrations. The new techniques introduced significant departures from the scope and intent of basic enzymatic theory. The mathematical notations for the enzyme kinetics have evolved from studies with clearly defined systems and the extent to which they apply to the measurement of uptake rates in complex natural water has not been demonstrated. Conversely the interpretation of "constants" derived from natural systems is problematical because both populations and substrates are heterogenous mixtures.

In aquatic environment the measurement of heterotrophic activity (defined as the utilization of dissolved organic matter) and bacterial biomass still presents some difficult problems. Plate counts have notably very little value in assessing this activity (Sorokin, 1971a), since media are different from those of the natural environment and the species developing in such media are not necessarily those which would be most active in the sea. Further only colony forming species are counted and the determination of microbial biomass is inaccurate. Seki et al. (1971) described a method for measuring the mineralisation rate in fresh water. The levels of added isotope employed by them are much higher and probably unsuitable for seawater.

Tritiated substances were used for studying the heterotrophy in seawater (Azam and Holm-Hasen, 1973) by the methods based upon radioactive tracers and microbiological techniques were introduced to measure

turn over times of dissolved sugars, amino acids and organic acids in aquatic biotopes (Parsons and Strickland (1961) Vaccaro and Jannasch (1966) Wright and Hobbie (1966); Allen (1969) Hamilton and Preslan (1970); Parson and Morita (1974). Seki et al. 1972, 1975, Gocke, 1975a; Carney and Colwell (1976). These studies have provided meaningful information about the incorporation and respiration of many organic compounds (Chiefly simple carbohydrates, monomers or aminoacids) in limnetic, estuarine or oceanic environments. However information about bacterial excretion of organic compounds after such substrate uptake is minimal. Nalewajko and Lean (1972); Nalewajko and Dunstall, (1976) studied on the uptake and turnover times of organic substances in different aquatic environments indicated that heterotrophic bacteria are the major agents that utilise these compounds with high efficiency. (Wright and Hobbie, (1966); Crawford (1970, 1974); Robinson, et al. (1973); Williams et al. (1976); Coughtan and Al-Hasan (1977) Hoppe (1978).

This activity of heterotrophic microbial population in natural waters is often determined by measuring the O_2 consumption rate or the uptake of ^{14}C labelled organic compounds. The O_2 consumption method involves the incorporation of a water sample in the dark at in situ temperature by applying suitable conversion factor the uptake rate of organic carbon can be calculated (Sorokin and Kadota, 1973). Qasim et al (1973) found that variations in the half-saturation constants (K_s) for both Biddulphia sinensis, Greville and Ceralium furea Ehrenberg gave an index of their capacity to utilise phosphate and nitrate when available either singly or in combination.

Parson and Strickland (1962) introduced the technique, for the measurement of heterotrophic activity in natural waters by applying ^{14}C labelled substrates, glucose and acetate. This technique was modified by Hobbie and Crawford (1969) which is widely employed now. The major difficulty in measuring heterotrophic activity in environments is that the concentration of the utilisable organic compounds are generally very low often in the order 10^{-9}, 10^{-8}M. Analytical procedures rarely sensitive enough to determine low concentrations, and also the conventionally used ^{14}C labelled tracers have to be added in relatively large amounts due to their low specific activity.

Bacterial growth rate and productivity were studied in sandy beach sediment and overlying water (Meyer Reil et al., 1978). Lake sediment (Tobin and Anthony, 1978), sea grass sediment (Moriarty, 1981; 1982; 1985a) marine surface (Fuhrman and Azam, 1982) water column and sediment of the Georgia Coast (Newell and Fallon, 1982) in Lake Erken (Bell, 1984) and swamp ecosystem (Murray and Hadson, 1984). Donald Edward Francisco (1970) observed in North Carolina, USA, that qualitatively and quantitatively changing bacterial community is responsible for the uptake of glucose and acetate and none of the environmental parameters studied influence of the concentration of viable bacteria. Gocke (1975a; 1977) studied short-term variations of heterotrophic activity and compared methods for determining the turn-over times of dissolved organic compounds in the Kiel Fjord. Hall et al. (1972) and Meyer Reil (1978) showed that the gross uptake of ^{14}C glucose by bacteria in disturbed sediment core

was approximately one order magnitude lower than uptake in disturbed cores. In the coast of South Africa a clear relation between primary production and heterotrophic activity was seen. An attempt was made by Hoppe (1976) to measure radio-isotopic incorporation of substrate by individual cells and found one cannot be certain whether label is incorporated without cell growth or whether an unlabelled cell is dead, resting or simply does not use the added substrate. Cavari et al. (1978) found rates as high as 1.0 mg/l/hr of glucose uptake in photic zone during October-November and at all depth during May-June.

Iturriaga (1979) found that organic particulates in the Baltic sea is transformed considerably during sedimentation. Decomposition of settled material resulted in a significant loss of organic matter which was temperature dependent. Uptake rate of added substrate provided important criteria for evaluating the different methods. Ansback and Blackburn (1980) found the rate constants for acetate turnover were higher at the sediment surface in the spring but did not vary seasonably at 4 to 6 cm depth.

Gocke et al. (1981) found that (1) not all of the dissolved free glucose (existing as truely free absorbed or complexed free glucose) is available to the heterotrophic micro-organisms (2) During the relatively short duration of the experiments the added ^{14}C-labelled glucose may not be proportionally distributed between the different fractions of the dissolved free glucose. Instead the fraction of the truly free glucose might be higher specifically labelled than the absorbed and complexed ones which may lead to an over-representation of the small fraction of

the truly free form. He also concluded using concentration of the dissolved free substance obtained by chemical analysis to calculate the flux might be misleading.

Autotrophic and heterotrophic activity measurements on the continental shelf of south eastern U.S. was done by Jacobsons (1981). Glutamate and glucose uptake, respiration rates and correlations suggested all these variables were interrelated and reflected relative levels of microbial heterotrophic activity. Nutrients and light appeared to be the controlling factor of photosynthesis and heterotrophic potential in Georgia coast (Jacobsons et al., 1983). Ferroni (1983) found turnover time for glucose increased with decreasing pH and V_{max} values decreased with decreasing pH. A significant positive linear relationship was found by Cavari (1984) between glucose uptake rates and $K_t + S_n$ values. Sang-Jin-Kim (1991) found extracellular activity in the Bransfield strait Antarctica and the turnover times ranged from 41-2094 hrs. Turnover time of the organic matters were extremely variable depending on the sampling station and water depth. Ducklow (1991)'s results suggest that the processes usually assumed to supply carbon that supports bacterial production provide only a fraction of the bacterial demand in the north western Indian Ocean.

Physio-Chemical relations

An aquatic microbial community is composed of an assemblage of highly diverse population. The elucidation and definition of the structures of the bacterial component of communities within estuaries and Oceanic ecosystems has been focussed by numerous investigations (Herbland et al., 1975; Bell., Franklin et al., 1980; King et al., 1980; Martin, 1979).

The effect of temperature on bacterial production has been studied by several workers (Lovell and Kropka, 1984; Pomeroy and Diebel, 1986; Newell and Floodgate,1971; Newell, 1969). Takashi and Ichimura (1971) found the influence of temperature on glucose uptake in marine waters. The need to control temperature under which the bacterial activity measurements are made and are stressed by Wright, R.T. (1984).

The correlation between heterotrophs and chemical parameters of sandy beach sediments in Kiel was studied by Meyer-Reil et al. (1978) and it was found that variation of organic and inorganic nutrients correspondent to variation of microbial activity and physio-chemical parameters. Chandrika (1984) found a highly significant relationship between the bacterial density and nitrite and phosphate in Cochin backwaters. Alaguravi (1984) found positive relationship between temperature and heterotrophic bacteria as well as sulphate reducers in the Pokkali field at Narakkal and in the perennial pond the pH, and Oxygen did not show any relationship with sulphate reducers.

Primary productivity

The primary production in the sea is one of the most fascinating problems in marine biological research. With the introduction of the radio active carbon isotope (^{14}C) in the study of marine productivity, investigations have gained greater significance and wide popularity among fishery scientists. No data were available on the production of organic matter until the Danish Galathea Expedition laid the foundation by the introduction of radio-active carbon (^{14}C) in the study of primary production

and made measurements in the equatorial part of the Indian Ocean and in the Bay of Bengal (Steeman Nielson; 1952, Steeman Nielson and Jenson, 1957). From the beginning it was recognised that during photosynthesis algae release some of their photosynthate into the medium as DOC and this material can be metabolised by microheterotrophs. In view of the importance of productivity studies in fisheries research, Prasad and Nair initiated the investigations in 1956 in the inshore waters of Mandapam. To begin with the well-known 'light and dark bottle technique' was used. Later with the consignment of carbon-14, the method yielded useful information on the production of organic matter and based on that, assessment of the potential resources in the inshore waters was made (Prasad and Nair, 1960, 1963). Photosynthesis and heterotrophic processes involving the release and subsequent uptake of dissolved organic material in sea water are ill-understood. Numerous algal and bacterial species can use glucose heterotrophically when present as free carbohydrates in the aquatic environment, (Vallentyn, 1957; Duursma, 1965) and can be traced to plant and animal excretions (Guillard and Wargusky, 1958; Fogg, 1958) and decomposition.

A variety of marine organisms, both algae and small crustacea (Lewin Provasoli and Shriaishi, 1959, 1963) have been reported to exist heterotrophically on soluable substrates to varying degrees. Most of the organic material found in the sea is in solution and consists in part of a wide variety of carbohydrates, lipids, hydrocarbons and aminoacids, plus complex molecules as vitamins, hormones etc. (Duursma, 1965). As with humic substances in soil the major fraction of the dissolved organic

material in the sea seems to have measurable microbial decomposition (Menzel, 1964). Glucose appears to meet the essential requirements of a useful index of heterotrophic potential in natural waters. A smaller fraction is assumed to consist of less stable compounds capable of supporting microbial transformation and growth. Prospects for progress in this area were considerably improved by Parsons and Strickland (1961) who applied some basic notation from enzyme kinetics to evaluate the relative heterotrophic potential of the water off British Columbia.

Dissolved organic carbon

There are strong relation between microbial activity and dissolved organic carbon. Evidence shows that there is a close coupling between planktonic bacteria and organic solutes. The total organic carbon pool is large when compared with the stock of bacteria. The open ocean euphotic zone, value of DOC may range from 0.6 to 1.0 mg C/l (Williams, 1975) the bacterial carbon content in this area is approximately 0.2 to 20/mgC/l assuming an average volume of 0.1 μm^3 for a bacteria (Ferguson and Rublee, 1976). The Chemo-organotrophic utilization of dissolved organic compounds by planktonic bacteria and algae in a pond was studied by Allen (1969). Seki (1970) studied the microbial action on particulate organic matter in the euphotic zone.

Bacteria are considered the major consumers of dissolved organic matter (Schelske & Odum, 1961). However bacteria are responsible for at least some of the additions of dissolved organic material in the sea.

Anderson et al. (1981) studied the inter relationship between heterotrophic bacteria carbon, nitrogen and Chlorophyll in an intertidal sediment. Free bacteria in the water have no source of nutrition other than dissolved organic substrates, while attached bacteria presumbly are utilizing particles both as habitat and substrate while utilizing dissolved material as well. The DOC is relatively refractory. All or some of this material is believed to be transformed by free living deep sea bacteria, which are slow growing psychrophiles, and this is reasonable in view of the fact that residence time of DOC in the ocean is orders in magnitude shorter than that of Na^+ or Cl^- (Williams et al., 1969) the fate of the labile DOC, although it has been generally assumed to be produced by phytoplankton (Fogg, 1977; Thomas, 1971; Nalewaiko, 1977). Additional DOC is produced during the transformation of faecal materials by bacteria and protozoans, most of which takes place in the upper 100 meters of the water column. A shift of attention from phytoplankton to zooplankton as producers of DOC may be appropriate while bacteria should be viewed both as producers and consumers of DOC (Fuhrman et al., 1980). Dynamics of bacterial decomposition and nutrients regeneration in the inshore waters off Cochin was studied by Chandrika, (1980).

Hobbie and Rublee (1977) demonstrated a close relationship between phytoplankton, primary production and potential bacterial uptake of organic substances.

The primary production and heterotrophic activity have been studied in the literal zone of a lake (Allen, 1973), Coast of North-West Africa

(Derenback, 1976) English Channel (Lancerot and Billen, 1984) and found the relationship between bacterial productivity and primary productivity was found highly significant. While studying the productivity of bacteria and micro algae in sediment of coral reef, Moriarty et al. (1985) observed that bacterial production was often a high proportion of primary production.

The further elucidation of the connection between primary production, exudation of organic substances by the phytoplankton and their uptake by heterotrophic micro organisms in aquaculture pond represents an important research aim for contemporary aquatic microbiology and more detailed information is made available by the present additional research in this direction.

II. MATERIAL AND METHODS

STUDY AREA

The present investigation was carried out at Narakkal (76°14'E Long; 10°03' N Lat.,), a fishing hamlet in Vypeen Island about 10 kilometres North-west of Cochin, Kerala (Fig.A). The land strip enclosed within the Arabian Sea on the western side and the Vypeen Channel, a branch of Cochin backwater system on the eastern side, is characterised by several low-lying fields interconnected by brackishwater canals. The canals are connected to the Cochin backwater system through a gut located at the mouth of the estuary, in the south and by another at Azhikode at the northern end of the island, which facilitate entry to tidal water. Two different types of prawn culture ponds were selected for the present study. Viz., a perennial prawn culture pond and a seasonal culture pond locally called 'POKKALI' field, are situated at Narakkal near Cochin.

Perennial pond is large and comparatively deeper where prawn culture is practised throughout the year. The perennial pond located within the premises of the prawn culture laboratory of the CIBA, Narakkal. The average depth of the perennial pond is about 1 metre. The pond is stocked with prawns and chanos.

Seasonal pond is an earthern field located further away from perennial pond which is used for culture of fish and prawns from December to May during which period, the saline nature of water is conducive for culture. The culture is wounded up by April when the field is utilised for cultivating a special variety of paddy called 'POKKALI' following

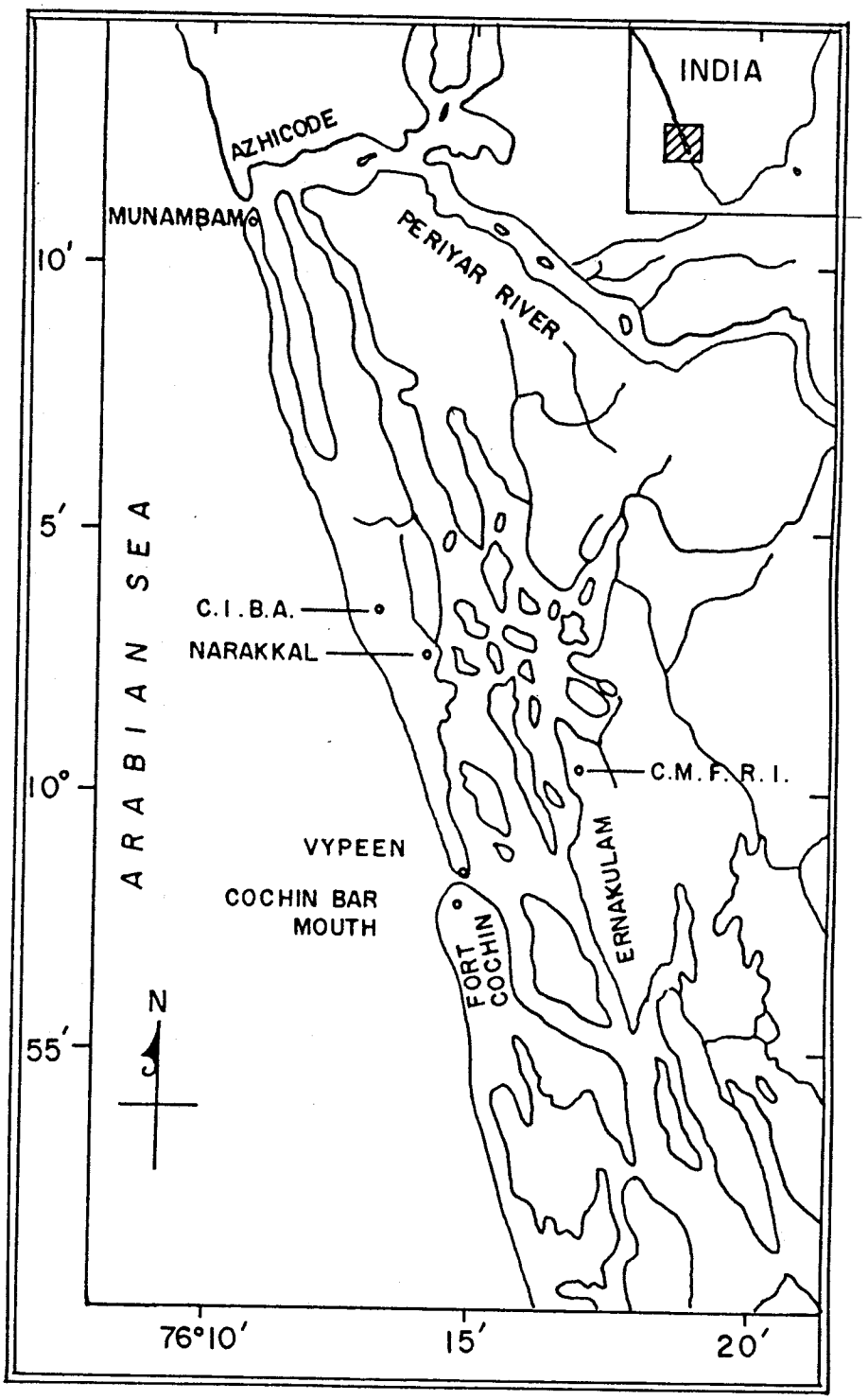

Fig.A. Map showing the sampling stations during 1989 and 1990.

the traditional method. The seasonal pond chosen was having an average depth of about 0.5 metre and it had an area about 0.4 ha. Though further away from perennial aquaculture system it is connected by the same feeder canal that supplies water to the other culture systems.

From these two stations water and sediment samples were collected at monthly interval for a period of two years (from January 1989 to December 1990) to study heterotrophic bacterial activity using tracer techniques. In addition hydrological parameters such as temperature, salinity, dissolved oxygen, pH, primary production, chlorophyll pigments and total aerobic population were collected. Samples are always taken at the same time of day (usually in the morning hours). Sample analysis must be made as soon as possible since the shorter the time between the collection and incubations, the more accurate the results.

Data were collected in seasonal pond only for 5 months in 1989 as paddy cultivation is practised during the rest of the period.

TRACER STUDIES

Sampling for tracer study

Water samples for tracer incorporation experiments were collected and transferred to 5 litre linear polyethlene dark carboys and transported to the laboratory within 3 hour period. Heterotrophic uptake studies were performed under sterile condition using C^{14}-substrates.

UPTAKE KINETICS IN ECOSYSTEM

Principle

Organic compounds are present in natural waters in low concentration and the usual bioassay methods are limited to vitamins and other micro-

nutrients. Radio active labelled glucose and acetate uptake is used to measure glucose and acetate concentration in natural waters but more importantly the curve relating uptake velocity to substrate concentration permits detection of two different types of mechanism of uptake. Specific transport systems effective at very low substrate concentrations, traced to the bacteria; and a diffusion mechanism effective only at higher substrate concentrations, to the algae. Standard techniques permits measurement of nutrient turnover rates and approximation of substrate concentration in an ecosystem. Thus velocity of uptake of substrate in nature can be used as a measure of heterotrophic activity. This application of organic radio-isotope methodology is useful particularly to those interested in dynamics of an ecosystems whether it is a pond, lake or sea.

Heterotrophic bacterial uptake Analysis

To determine the turnover time (T_t) due to active transport (hrs), T_t - turnover time due to velocity ($\mu gC\ l^{-1}h^{-1}$) the maximum velocity uptake (V_{max}) and the sum of the transport "constant" and the natural substrate concentration ($K_t + S_n$), the method of Parson and Stricklard (1962) was used, which was further improved by Wright and Hobbie (1966). Some modifications were introduced which are given in detail by Gocke (1975, 1977).

For the assessment of the microbial activity a known amount of radio active glucose and acetate is added to a sample of water, and

incubated at the in situ temperature in the dark. After incubation the incorporated radio activity taken up by bacteria in a given time are measured with a suitable Scintillation counter.

Special apparatus

(i) 25 mm diameter manifold filtering unit, fitted with funnel to hold the entire water sample.

(ii) 25 mm Sartorius filter paper (Pour Size 0.2 μ) were imported from WEST GERMANY.

(iii) Scintillation Vials (ECIL), suitable for dissolving millipore filters and with low quenching in the presence of small amounts of water (20 ml capacity).

(iv) Scintillation counter (LSS-20) (ECIL) (Plate - I).

Special reagents

The two tracers selected for the present study were radio active-D-Glucose-C^{14} (U); and Sodium acetate - 1-C^{14}(U), were purchased from ISOTOPE GROUP, BHABHA ATOMIC RESEARCH CENTRE, TROMBAY, BOMBAY - 400 085, INDIA.

Scintillator Components

1-4- Dioxane (p-Dioxan) - $C_4 H_8 O_2$ Scintillation grade,

1-4- Bis (5-phenyloxazol-2-YI) Benzene (POPOP)

$C_{24} H_{16} N_2 O_2$

Napthalene $C_{10} H_{16}$

these primary and secondary Scintillators were procured from SISCO Research Laboratories Pvt. Ltd., Bombay - 400 060.

Scintillation solvents play an important role in determining the Scintillator efficiency. An efficient solvent facilitates both energy conversion and energy transmission.

In selecting the Scintillation solvent optical transparency to the photons emitted by the solutes is essential. The solvent should have minimum toxicity and high flash point in order to minimise health and fire hazards. It must possess adequate solubility for the solute and the specimen so that an efficient homogenous sample can be prepared.

In the present observation p-dioxane was used as a solvent because of its water miscibility and allows specimen to be incorporated into the Scintillation system. Naphthalene at a concentration of 50-100 g/l was selected as intermediate and secondary solvent as it facilitates the solvent to solute excitation energy transfers in liquid scintillator containing p-dioxane as the solvent. The aliphatic solvent p-dioxane was used because of its water misibility which allows aqueous specimen to be incorporated into the Scintillation system. Quenching by dissolved oxygen in these aromatic solvents is significant and for getting higher flurescence nitrogen gas was bubbled in the vial for higher field of fluorescence. Excited naphthalene molecules are less prone to impurity quenching and result in an increase light output of the Scintillator.

LIQUID SCINTILLATION COUNTING

The liquid Scintillation system works on the photonmission and its subsequent conversion to electronic pulse, the former being released by certain compounds when bombarded by nuclear radiation.

1. Determination of counting efficiency

The activity of the quenched standards of ^{14}C was determined by two channel counting and the channels ratio (AB/AC) was computed. From this the counting efficiency was worked out as presented in table I.

The counting efficiency was plotted against channels ratio to obtain the quench correlation curve (Fig.2).

Quench correction in liquid scintillation system

The liquid Scintillation process is based upon the conversion of a part of kinetic energy of an ionizing particle into photons which are collected by photomultiplier tubes and subsequently summed, sorted and counted.

Any process which interferes in the creation or transmission of light from the solution is called quenching. Impurities in the specimen as well as the liquid scintillation itself, quench the available energy reducing both the pulse height and the total number of detectable events.

In the present experiment the channel ratio method of quench correction is described for counting chemically quenched ^{14}C samples. The dpm of a quenched sample is also verified by using internal standard method.

Table 1. The count rate and channels ratio for the Quenched Standards of C^{14}

Quenched Standards	C_1	C_2	C_2/C_1	$C_1/203000 \times 100$
1	1,72,182	20564	0.119	84
2	1,50,664	35916	0.23	74
3	1,06,933	49592	0.46	52
4	68,294	45910	0.67	33
5	33,738	28494	0.84	16
6	19,629	17811	0.90	9
7	11,521	10971	0.95	5
8	8,206	7946	0.96	4

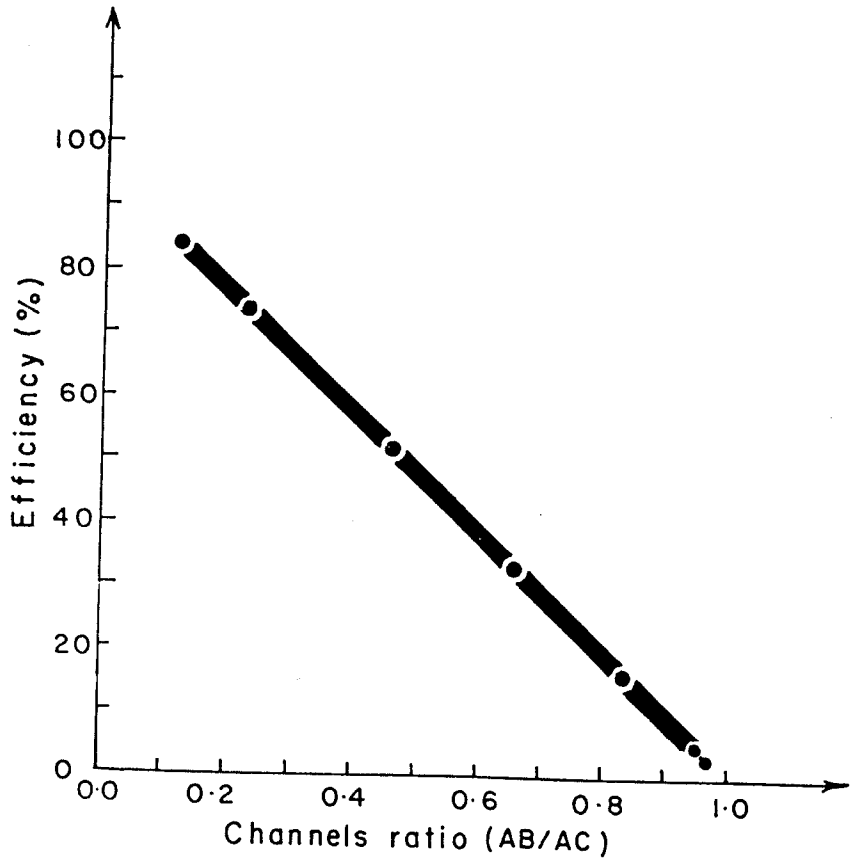

Fig. 2. Channels ratio versus detection efficiency of the counting system.

In the internal standard method after counting the sample, a known quantity of reference standard is added to the same vial and counted. The increase in count is then attributed to the added standard, from which the efficiency of the quenched sample is calculated. It can be reasonably assumed that the standard doesn't significantly contribute to quenching.

Precautionary Measures

Usually in radio active work glasswares must be washed with a special cleaning mixture either a solution of sodium bicarbonate or polianski mixture, (water - 10 litres, concentrated nitric acid 675 ml, Saturated Exalic acid - 1 litre, Sodium Chloride - 200 g). When working with C^{14}, the glasswares must be washed preferably with sodium carbonate solution. The glasswares were then washed in the usual manner after cleaning initially with saturated solution of sodium carbonate in the present observation.

PROCEDURE

The procedure involves the addition of different amounts of U-^{14}C-labelled substrate to identical sub-samples. The substrates used for heterotrophic analysis was D-Glucose-C-14(U) ($HOCH_2CHOH_4CHO$) in 80% ethanol with specific activity of 160 mci/m mole and Sodium acetate-1-C-14 (CH_3COONa) a specific activity of 57.8 mci/m mole (ISOTOPE GROUP: BARC, BOMBAY).

The addition of substrate was according to the pattern described by Wright and Hobbie (1966) and the uptake of uniformly labelled ^{14}C-glucose was measured at four different concentrations. The usual

additions were 10,20,50 and 100 µl in duplicate and one 20 µl addition to the killed blank. The final concentration ranges of glucose, sodium acetate were 0.58 µgC l^{-1} to 2.32 µgC l^{-1}, 0.22 µgC l^{-1} to 2.175 µgC l^{-1} respectively. 20 sample bottles were used for each depth and for each substrate. Duplicate sub-samples were used at each of four different concentrations. Labelled substrates were pipetted into 50 ml samples taken in amber coloured borosil reagent bottles which were prefiltered through a 25 µ mesh bolting silk. A fifth sample with 20 µl - fixed immediately at the beginning of the experiment with Lugol's iodine as a control.

Composition of the Lugol's solution

Iodine	-	5.0 gms
KI	-	10.9 gms
Na $C_2H_3O_2$	-	15.5 gms
H_2O	-	1000 ml

The samples and the blanks were then incubated at the in situ temperature in the dark for 0.5 hours depening upon the activity of the population. After incubation further metabolism was stopped by the addition of 5 drops of neutral Lugol's per sample. Leakage of C^{14} from the cells was found to be insignificant if held for period of less than 6 hours before filtering. The samples were filtered using a manifold filtering unit capable of simultaneous filtration of six samples at once through 0.2 µ membrane filter (Sartorius) and the trapped cells on the filter were measured with

three 10 ml portions of filtered cold pond water. Suction pressure applied was 1/3 of an atmosphere during filtration. The filter paper was removed from the holder while maintaining the vacuum and placed in scintillation vials containing 10 ml of dioxane based scintillation flour (SISCO Laboratories, Bombay), after gentle shaking the sealed vials were kept overnight in dark and then the vials were counted in scintillation counter at window values appropriate for ^{14}C. The uptake kinetics were determined using Line Weaver-Burke modification of the Michaelis - Menten equation.

The following relationship was used to calculate the various kinetic parameters.

$$(C\,\mu t)/c = (K_t + S_n)/V_{max} + A/V_{max}$$

Where,

C = is the activity of CPM of 1.0 μ ci of the substrate in the particular counter used.

μ - is the number of μci added

t - is the incubation time (hr)

c - is the radioactivity of filtered organisms (CPM)

$(K_t + S_n)$ - K_t - is an uptake of constant analogous to the michaelis constant, after determining the relative uptake at various concentrations of added substrate.

S_n - in general, could not be measured in natural waters because of the limits of analytical methods.

V_{max} - is the minimum uptake velocity in $\mu g\ l^{-1} h^{-1}$

Using this the uptake measurements at several low concentrations of added substrate were plotted with C μt/c on the ordinate and A on the abscissa (Fig.1). Thus the slope is - (K_t + S_n). The intercept on the ordinate is S_n/V which equals the turnover time, (T_t) i.e. the time in hours required by the natural microbial population to utilise the natural substrate uptake were converted to best fitting straight line using least square analysis and a modified Lineweaver - Burk equation. The parameters calculated are related to the net uptake of substrate because respiration studies were not included in the present investigation. This means the gross uptake V_{max} would be higher and T_t shorter, K_t + S_n is not affected.

HYDROLOGICAL STUDIES

From two fixed stations water samples were collected and analysed for temperature, salinity, dissolved oxygen, pH and chlorophyll pigments. Sediment samples were analysed for temperature and pH. Water samples were collected in 250 ml poplypropylene bottles for salinity and pH. For estimation of chlorophyll, samples were collected in 2 litre wide mouthed polypropylene bottle. Before drawing, the sample bottles were precleaned twice with the ambient water. The samples were preserved in an ice box during transportation till they were analysed.

After reaching the laboratory water and sediment samples were analysed for various parameters within 3 hours of collection.

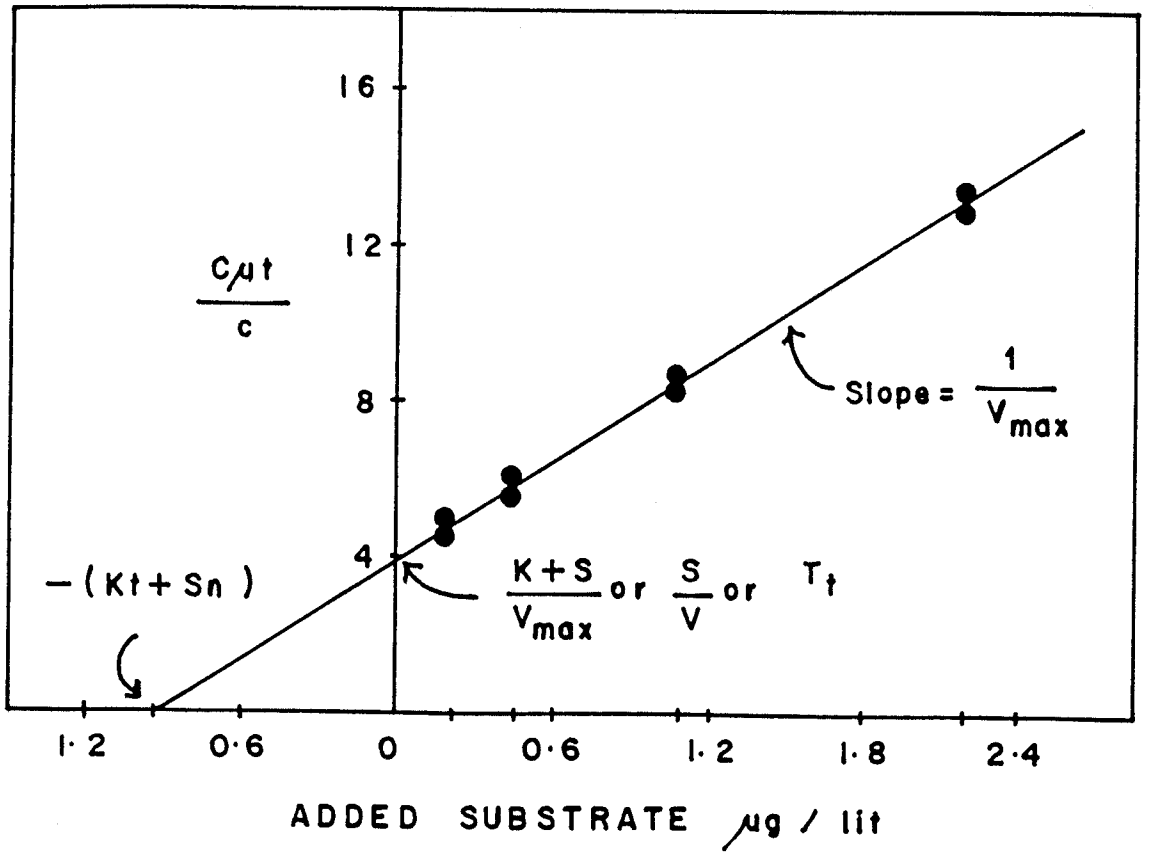

Fig. 1. Uptake of acetate by the natural population plotted according to Equation-5, sample on 14.12.1989 from surface water.

MEASUREMENT OF HYDROLOGICAL PARAMETERS

TEMPERATURE

Temperature was determined immediately after collection of water and sediment samples at the prawn culture fields with an accuracy of ± 0.1°C using precision mercury thermometer.

SALINITY

Salinity was estimated by the classical Mohr titration (Strickland and Parsons, 1968). The outline of Mohr-Knudson method is as follows:

Ten millilitres of water samples were titrated against the silver nitrate solution with potassium chromate as an indicator. Care was taken to arrive at the exact end point colouration in all the samples and every set of titration, silver nitrate was standardised using standard seawater supplied by the Oceanography Institute, Copenhagen. Each sample was titrated and the mean values was taken. Salinity of the sample was calculated using the following formula.

$$\text{Salinity } (‰) = \frac{V_1 S}{V_2}$$

Where

V_1 = Volume of silver nitrate for 10 ml Standard seawater.

V_2 = Volume of silver nitrate for 10 ml sample

S = Salinity of standard seawater

DISSOLVED OXYGEN

Dissolved oxygen samples were collected using 125 ml 'corning' bottle with BOD stopper. Traditional Winkler method with azide modification

was used for the determination of dissolved oxygen content (Anon, 1975). The outline of this method is as follows:(Claude & Pillai (1984).

To a sample in 125 ml bottle, 2 ml of manganous sulphate solution and 2 ml of alkali iodine-azide solution were added. The bottle was stoppered with care to prevent air bubbles. The solution was mixed by inverting the bottle several times. The precipitate was allowed to settle and dissolved in the laboratory using 2 ml of concentrated sulphuric acid.

From this, 100 ml of sample was taken for titration and was poured into a 250 ml beaker, titrated with standard sodium thiosulphate (6.3 g/L) solution to a pale straw colour. About 5 drops of starch indicator solution was added to this and titrated until blue colour disappears. The following equation was used to calculate the dissolved oxygen concentration.

$$\text{Dissolved oxygen (mg/L)} = \frac{(T).\ (N).\ (8{,}000)}{S}$$

Where,

T = Volume in millilitre of sodium thiosulphate

N = Normality of sodium thiosulphate

S = Volume in millilitres of sample

HYDROGEN-ION-CONCENTRATION (pH)

Electrometric method with electrically operated Elico-pH meter having a glass electrode and calomel electrode was used for determination of hydrogen ion concentration values with greater accuracy.

Water samples collected in 250 ml polyprophylene bottles were used for the determination of hydrogen ion concentration. The instrument

was calibrated with the help of pH buffers. After taking pH meter reading, the in situ pH was calculated using the formula (FAO, 1975).

$$\text{pH in situ} = \text{pH measured} + 0.0118\ (t_2 - t_1)$$

Where,

T_1 = Temperature in situ

T_2 = Measured temperature

PRIMARY PRODUCTION

Another of the parameters thought to be related to heterotrophy is primary productivity.

Primary production was estimated by measuring release of oxygen which dissolves into the surrounding water resulting in increase of oxygen during photosynthesis and can be computed by measuring the dissolved oxygen at the beginning and at the end of the incubation period.

Oxygen technique (Gaar and Garder, 1927) was used for the estimation of primary production. In this method, composite samples were categorised into three groups viz., initial bottle (IB), light bottle (LB), and dark bottle (DB). All the three were ordinary corning bottle, but the last one was painted black and wrapped in aluminium foil.

Initial dissolved oxygen concentration was determined by fixing the initial bottle with Winkler A and B. The light and dark bottles were kept in the lab under flourescent light for a period of 3 hrs. In the dark bottle only respiration takes place, whereas in the light bottle both photosynthesis and respiration take place. The difference in the oxygen

content between light and dark bottle was taken as gross production and the differences between light and initial bottles were taken as net production. Production was calculated as follows:

$$\text{Production (mgC)} = \frac{O_2 \text{ (mg)} \times 0.375}{PQ}$$

Where,

PQ (Photosynthetic quotient) is taken as 1.25. Assuming that photosynthesis has taken place 10 hrs a day, then primary production per day can be calculated as follows:

$$\text{Primary production (mgC/m}^3\text{/day)} = \frac{O_2 \text{ mg} \times 0.375 \times 1000 \times 10}{1.25 \times A}$$

Where,

A = Number of hours of incubation

CHLOROPHYLL AND CAROTENOID DETERMINATION

A method followed by Jeffrey and Humphrey (1975) as described by Parsons et al. (1984) was used for chlorophyll and carotenoid estimation. A known volume of sample was poured into a millipore filtering equipment containing a memberane filter paper. Sample was filtered under 1/2 atmosphere pressure vacuum. To this 3-5 drops of Mg Co_3 solution was added while filtering. Filter was drained thoroughly and was placed in a 15 ml glass vial. To this 10 ml of 90% acetone was added. The contents of each tube was centrifuged for 5-10 minutes at 2000 rpm. The supernatant solution was decanted into the spectrophotometer cell and extinction was measured at the following wave length. Each extinction was corrected

a small turbidity blank by substracting the 750 nm from 664, 647 and 630 nm absorption. The 510 nm and 480 nm absorbance were corrected by substracting the 2X and 3X 750 nm absorbance respectively. The amount of pigment in the original sample was determined using the equation given below:

For Chlorophylls:-

(Ca) Chlorophyll-a = $11.85\ E_{664} - 1.54\ E_{646} - 0.08\ E_{630}$

(Cb) Chlorophyll-b = $21.03\ E_{647} - 5.43\ E_{664} - 2.66\ E_{630}$

(Cc) Chlorophyll-c = $24.52\ E_{630} - 1.67\ E_{664} - 7.60\ E_{647}$

For plant Carotenoids:-

(Cp) = $7.6\ E_{480} - 1.49\ E_{510}$

Where,

E stands for absorbance at different wave lengths obtained and Ca, Cb, Cc and Cp are the amount of chlorophyll a, b, c and carotenoids (µg/ml) if a 1 cm light path cuvette is used.

Then

$$\text{mg Chlorophyll or Carotenoid/m}^3 = \frac{C \times V}{v \times 10}$$

Where,

V = Volume of sample in litre

C = is the substituted value for Ca, Cb, Cc and Cp in the above equation.

v = Volume of acetone in ml (µg/l = mg/m^3)

INVESTIGATIONS ON AEROBIC HETEROTROPHS

COLLECTION OF WATER SAMPLES FOR BACTERIOLOGICAL OBSERVATION

Surface water was collected in sterile 300 cc glass bottles in aspectic condition and kept at 4°C until the time of bacteriological investigations in all the collection. Sediment samples were collected by the bottom grab (Vanveen grab 0.048 m^2, Barnett, 1959), for microbiological investigations the only suitable equipment for sediment collection is that one in which the cover of the grab can be opened from above. Investigations have shown that the number of bacteria and diversity of constituent groups decrease rapidly as the lower layers of the sediments are reached. Therefore, the top most layer (in particular, the sediment water interface) should be sampled for microbiological analyses. The sample bottles and polythene bags with sediment are held at 4°C until processing, 18-24 hrs later.

CULTURE METHODS

Synthetic media was used for culture of heterotrophic bacteria. Aged seawater was used in the preparation of media for heterotrophic aerobes. The pH of the media was adjusted to 7.2 respectively with the help of N/10 NaOH and N/10 HCl solution.

SEAWATER AGAR (SWA) FOR HETEROTROPHIC BACTERIA

Composition:

Peptone	-	1%
Ferric phosphate	-	1 pinch

Agar	-	2%
Aged Seawater	-	100 ml
pH 7.2 15 lbs	-	30 minutes

Seawater agar media was used to isolate all heterotrophic bacteria from water as well as sediment samples.

Cleaning and Sterilisation

Glasswares were first cleaned with detergents and then with acidified potassium dichromate ($K_2Cr_2O_7$) solution. They were thoroughly washed under running tap-water and finally rinsed with distilled water and left for drying.

The culture media was sterilised at 15 lbs pressure for 15 minutes in the autoclave. Petridish pair and pipettes were sterilised keeping them inside their respective cans in hot air oven for $1\frac{1}{2}$ hours at 160°C. Spatula was sterilised by dipping in rectified spirit and by flaming it to red heat.

PLATING

Quantitative analysis

Individual marine microbiologists utilise different methods to estimate bacterial population in a given sample. Every method has its advantage and disadvantages and there is a definite protocol for each method. The method utilised in marine microbiology are discussed in several papers and books (Jannasch, 1965; Rodina, 1972; Zobell, 1946).

Pour-plate technique

Depending on the anticipated bacterial numbers and the turbidity, samples were either concentrated or diluted. Plate counts were made by the pour plate technique using a medium composed of 0.1% yeast extract, 1% peptone and 2% agar in aged seawater. The medium was found to yield higher total counts than ordinary seawater without yeast extract or nutrient agar (Hi-media) in seawater but it required a standard incubation for one week at room temperature.

The bottles are vigorously agitated, the mouths of the bottles were flamed and a diluted series was prepared by serial dilution technique. 1 ml of the sample was added to 9 ml sterilised seawater and the test tube is agitated vigorously. The number of bacteria per millimetre has now been diluted to 1/10 (= 10^{-1}). This step is repeated with each of nine new sterile pipette until the anticipated number of bacteria per millilitre is less than 300. An attempt was made to have no more than 300 colonies per plate, as reciprocal influence will otherwise develop.

If a series of dilutions are made of an inoculum of a particular dilution/dilutions is pour-plated, the total number of viable organisms present in a given quantity of the dilution may be determined. This method is known as the pour plate method for the enumeration of viable organisms.

Calculation

Averaged the counts obtained and reported as aerobic plate count/ml/ gm. Total plate count per gm of the sample was computed as follows:

$$\text{No. of bacteria/gm/ml} = \frac{\text{No. of colonies/ml/gm x Reciprocal of dilution x 1}}{\text{Weight of the sample in gms}}$$

Statistical analysis

The results obtained during the present investigation were processed statistically to obtain mean and standard deviation of water and sediment for the perennial and seasonal ponds. Results of computer analysis of one-way ANOVA were used to test the significance of effect of seasons and also between surface and bottom water on various parameters. Correlation analysis was also carried out (Snedecor and Cochran, 1967) to understand the interrelationship between the different parameters. All data collected on the above aspects are given either in the form of graphic intensity charts or tables for effective presentation of the results.

III. RESULTS

PART - 1 KINETIC PARAMETERS

Seasonal changes in the kinetic parameters in surface and bottom water at perennial pond such as turnover time (T_t), maximum velocity of uptake (V_{max}) and the sum of transport 'constant' the natural substrate concentration ($K_t + S_n$) of glucose during 1989 and 1990 are presented in Tables 2 and 4, Figures 3, 5 and 7.

Results of observation on the kinetic parameters in the seasonal pond which was limited to the pre-monsoon season of 1989, and for three seasons in 1990 are presented in Tables 3, 5 and Figures 4, 6 and 8.

I. Results of glucose uptake experiments:

(a) Turnover time of glucose: T_t (hrs)

(i) Perennial Pond

The results (Fig.3) show that in surface water, during 1989, T_t varied from 3.445 hrs to 16.46 hrs, and in 1990 it ranged between 3.45 hrs and 17.14 hrs. Higher turnover time was recorded in April and September during 1989 and in 1990 it was from January to March and July to October. The turnover time was short during February and August in 1989 and May in 1990.

The seasonal mean of T_t for surface water was maximum during post-monsoon and minimum recorded in pre-monsoon (Table 2).

In bottom water T_t was generally higher and found to range from 5.22 hrs in December and 18.325 hrs in April during 1989. In 1990 T_t

Table 2. Seasonal mean and standard deviation for kinetic parameters of glucose in surface and bottom water at perennial pond.

Kinetic Parameters	Seasons		
	Pre-monsoon	Monsoon	Post-monsoon
PERENNIAL POND:			
Surface Water			
T_t (hrs)	6.98 ± 5.67	9.48 ± 3.58	11.13 ± 3.81
V_{max} ($\mu gC\ l^{-1}h^{-1}$)	0.45 ± 0.39	0.38 ± 0.31	0.31 ± 0.26
K_t+S_n ($\mu gC\ l^{-1}$)	3.47 ± 2.55	2.93 ± 1.77	2.80 ± 1.54
Bottom Water			
T_t (hrs)	10.19 ± 4.27	12.13 ± 1.86	8.85 ± 3.47
V_{max} ($\mu gC\ l^{-1}h^{-1}$)	0.27 ± 0.34	0.202 ± 0.203	0.10 ± 0.03
K_t+S_n ($\mu gC\ l^{-1}$)	2.21 ± 2.12	2.37 ± 2.33	0.98 ± 0.66

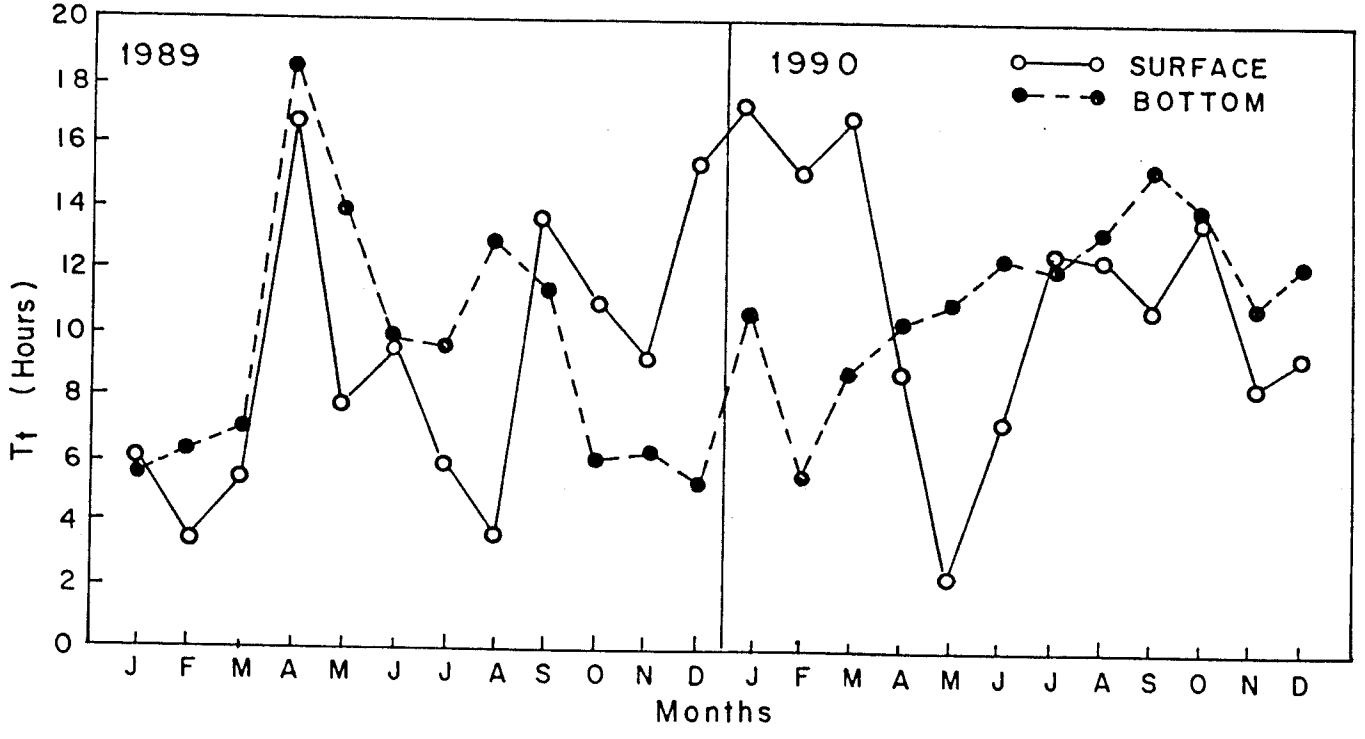

Fig. 3. Monthly variations in the turnover time of glucose in surface and bottom water at perennial pond, Narakkal, 1989 and 1990.

ranged between 5.724 hrs in February and 15.28 hrs in September. Higher T_t was recorded during April to September in 1989 and in 1990 it started April onwards upto December, shortest T_t of 5.724 hrs was recorded in February.

Seasonal mean for T_t in bottom water was minimum during post-monsoon (8.85 ± 3.47) and maximum was (12.13 ± 1.86) obtained during monsoon (Table 2).

The results of ANOVA for surface and bottom water over different seasons (Table 17 A & B); between surface and bottom water implies no significant variation for the T_t of glucose ($P > 0.05$).

(ii) Seasonal Pond

In seasonal pond the turnover time for the pre-monsoon season during 1989 ranged between 2 hrs in February and 15.45 hrs in April. Higher T_t was recorded during April and May (Fig.4). In 1990 the turnover time ranged from 8.02 hrs to 15.6 hrs. The shorter T_t was recorded during onset of pre-monsoon, monsoon and post-monsoon months (April, August and November). The higher turnover time range continued in March, May and June in surface water, whereas in bottom water the highest range was 10.375 hrs in May '89 and 15.54 hrs in February '90. The lowest range of turnover time was only 3.31 hrs in December 1989 and 3.308 hrs during March 1990.

Maximum seasonal mean was obtained during monsoon and minimum occurred in post-monsoon for surface water whereas in bottom water

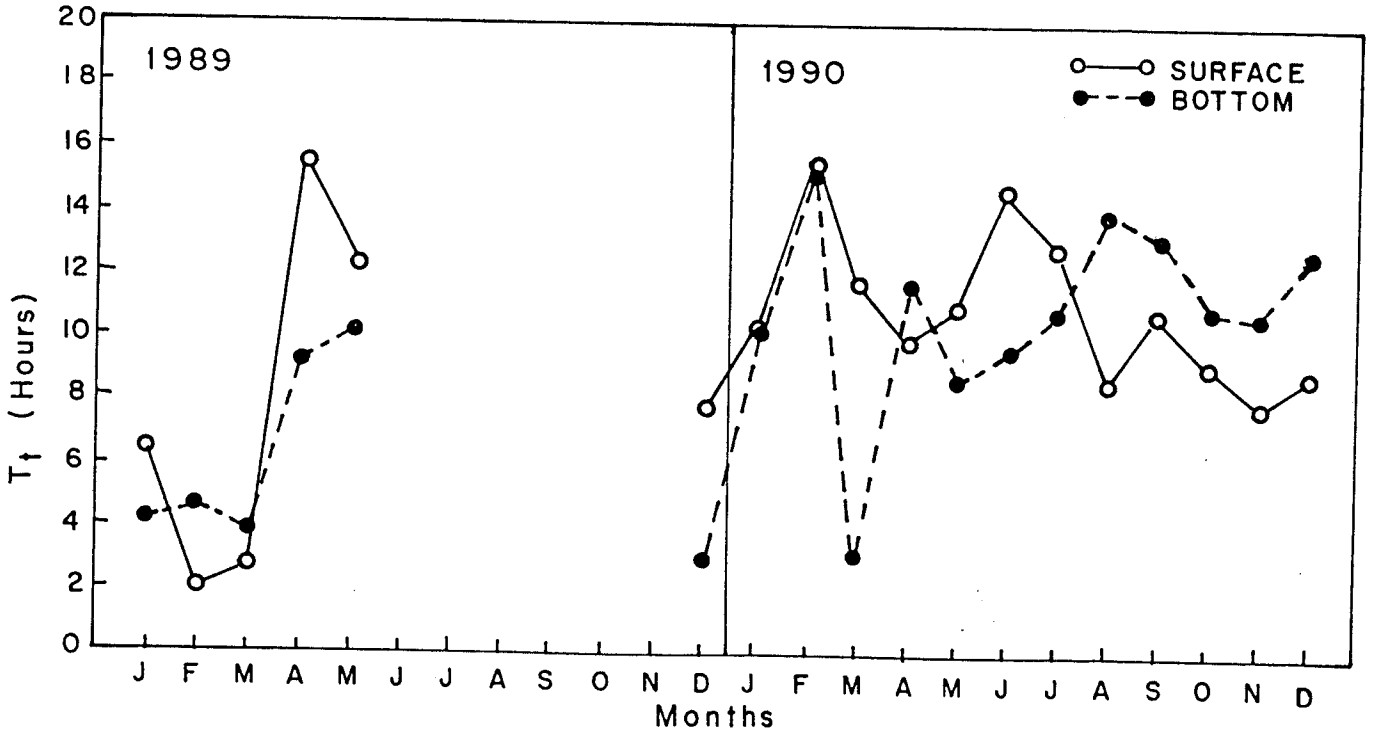

Fig. 4. Monthly variations in the turnover time of glucose in surface and bottom water at seasonal pond, Narakkal, 1989 and 1990.

Table 3. Seasonal mean and standard variation for Kinetic parameters of glucose in surface and bottom water at seasonal pond.

Kinetic Parameters	Seasons		
	Pre-monsoon	Monsoon	Post-monsoon
SEASONAL POND			
Surface Water			
T_t (hrs)	10.12 ± 5.18	11.76 ± 2.61	8.44 ± 1.34
V_{max} ($\mu gC\ l^{-1}h^{-1}$)	0.02 ± 0.14	0.19 ± 0.08	0.18 ± 0.11
K_t+S_n ($\mu gC\ l^{-1}$)	1.74 ± 1.08	2.07 ± 0.39	1.03 ± 0.13
Bottom Water			
T_t (hrs)	8.45 ± 4.25	11.89 ± 2.05	8.64 ± 3.82
V_{max} ($\mu gC\ l^{-1}h^{-1}$)	0.44 ± 0.43	0.09 ± 0.013	0.18 ± 0.13
K_t+S_n ($\mu gC\ l^{-1}$)	2.56 ± 1.58	1.21 ± 0.15	1.11 ± 0.72

Table 17. One-way ANOVA for turn over time of glucose in surface and bottom water of perennial and seasonal ponds during different seasons.

	SOURCE	DF	SS	MSS	F	REMARKS
	PERENNIAL POND					
A.	Seasons	2	12.942	6.471	0.33	NS
	Error	21	417.143	19.864		
	Total	23	430.085			
B.	Seasons	2	43.519	21.759	1.93	NS
	Error	21	236.565	11.265		
	Total	23	280.083			
	SEASONAL POND					
C.	Seasons	2	27.010	13.505	0.93	NS
	Error	15	217.562	14.504		
	Total	17	244.572			
D.	Seasons	2	35.156	17.578	1.24	NS
	Error	15	212.586	14.172		
	Total	17	247.742			

A & C - Surface Water, B & D - Bottom Water

NS - Not Significant

the seasonal mean was to be minimum during pre-monsoon and maximum was obtained in monsoon (Table 3).

Like perennial pond no significant variation was noticed over different seasons (Table 17 C & D); and (Table 30 D) between surface and bottom water for the T_t of glucose ($P > 0.05$).

(b) Maximum uptake velocity of glucose: V_{max} ($\mu gC\ l^{-1}h^{-1}$)

(i) Perennial Pond

The maximum uptake velocity (Fig.5) tended to increase during March and in July 1989 in surface water, the values being 1.247 $\mu gC\ l^{-1}h^{-1}$ and 1.972 $\mu gC\ l^{-1}h^{-1}$. V_{max} of glucose ranged between 0.1186 $\mu gC\ l^{-1}h^{-1}$ to 1.247 $\mu gC\ l^{-1}h^{-1}$ in 1989 and in 1990, 0.0778 $\mu gC\ l^{-1}h^{-1}$ to 0.471 $\mu gC\ l^{-1}h^{-1}$ respectively.

The V_{max} was somewhat constant from January to July and higher velocity of uptake were encountered in August (0.471 $\mu gC\ l^{-1}h^{-1}$ and October (0.394 $\mu gC\ l^{-1}h^{-1}$) during 1990.

Two peaks are evident in bottom water the primary peak falls in March 1989 (1.008 $\mu gC\ l^{-1}h^{-1}$) and secondary peak occurred in August 1989 (0.688 $\mu gC\ l^{-1}h^{-1}$) (Fig.5). The value for V_{max} of glucose was constant during April to July and again extends upto August 1990 with only slight variations. In bottom water the highest value of V_{max} 0.2431 $\mu gC\ l^{-1}h^{-1}$ was encountered, and the lowest V_{max} of glucose was observed during February, March, August and November 1990, the lowest value being 0.0964 $\mu gC\ l^{-1}h^{-1}$ respectively.

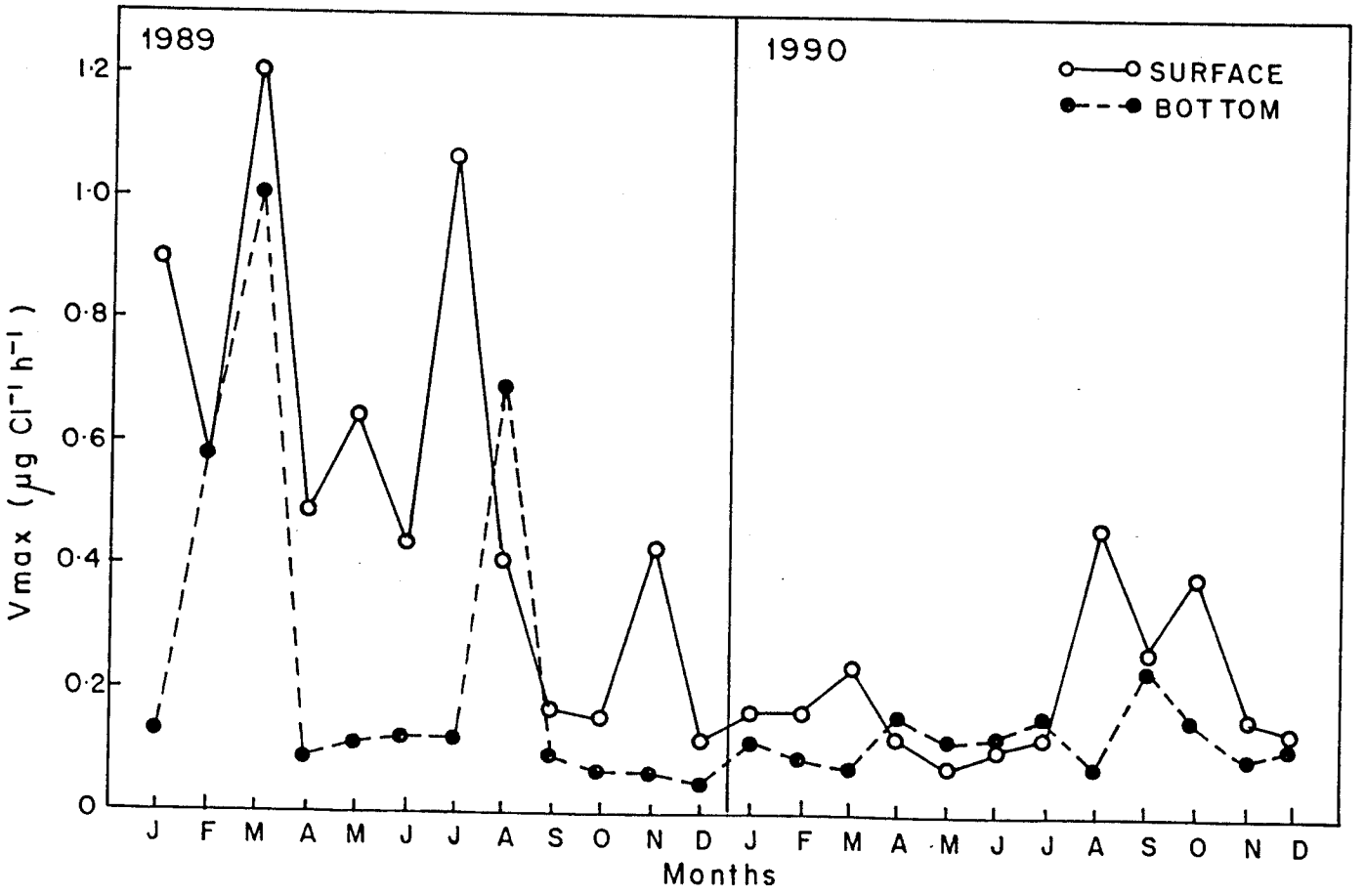

Fig. 5. Monthly variations in the maximum velocity of uptake of glucose in surface and bottom water at perennial pond Narakkal, 1989 and 1990.

For the V_{max} of glucose in surface and bottom water the maximum seasonal mean was obtained during pre-monsoon and minimum was encountered in post-monsoon (Table 2).

Analysis of variance over different seasons for surface and bottom water (Table 18A & B) showed no significant variation ($P > 0.05$); between surface and bottom water (Table 30 B) indicates significant variation for V_{max} of glucose ($P < 0.05$). In surface water the V_{max} of glucose concentration was found high.

(ii) Seasonal Pond

In surface water the lowest V_{max} of glucose was 0.09 $\mu gC\ l^{-1}h^{-1}$ observed during May 1989 and the highest V_{max} of glucose being 0.4395 $\mu gC\ l^{-1}h^{-1}$ was recorded in February 1989 (Fig.6). During 1990 highest V_{max} of glucose 0.316 $\mu gC\ l^{-1}h^{-1}$ was recorded in August and the lowest being 0.087 $\mu gC\ l^{-1}h^{-1}$ in January, whereas in bottom water the highest peak of V_{max} of glucose was observed in February 1989, the value being 1.36 $\mu gC\ l^{-1}h^{-1}$ during April 1989 and 0.2043 $\mu gC\ l^{-1}h^{-1}$ in December 1989. In 1990, it was found to be somewhat constant during monsoon and pre-monsoon months with less variation. 0.0509 $\mu gC\ l^{-1}h^{-1}$ was the lowest value recorded in March 1990 and highest was obtained 0.34 $\mu gC\ l^{-1}h^{-1}$ during May 1990.

In surface water the seasonal mean for the V_{max} of glucose was maximum during monsoon season and the minimum was in pre-monsoon, whereas in bottom water the highest seasonal mean was obtained in pre-monsoon and the lowest was observed in monsoon (Table 3).

Table 18. One-way ANOVA for maximum velocity of uptake of glucose in surface and bottom water of perennial and seasonal ponds during different seasons.

	SOURCE	DF	SS	MSS	F	REMARKS
	PERENNIAL POND					
A.	Seasons	2	0.079	0.039	0.37	NS
	Error	21	2.232	0.106		
	Total	23	1.225			
B.	Seasons	2	0.124	0.062	1.18	NS
	Error	21	1.102	0.052		
	Total	23	1.225			
	SEASONAL POND					
C.	Seasons	2	0.006	0.003	0.21	NS
	Error	15	0.214	0.014		
	Total	17	0.219			
D.	Seasons	2	0.399	0.199	2.11	NS
	Error	15	1.414	0.094		
	Total	17	1.812			

A & C - Surface Water, B & D - Bottom Water

NS - Not Significant

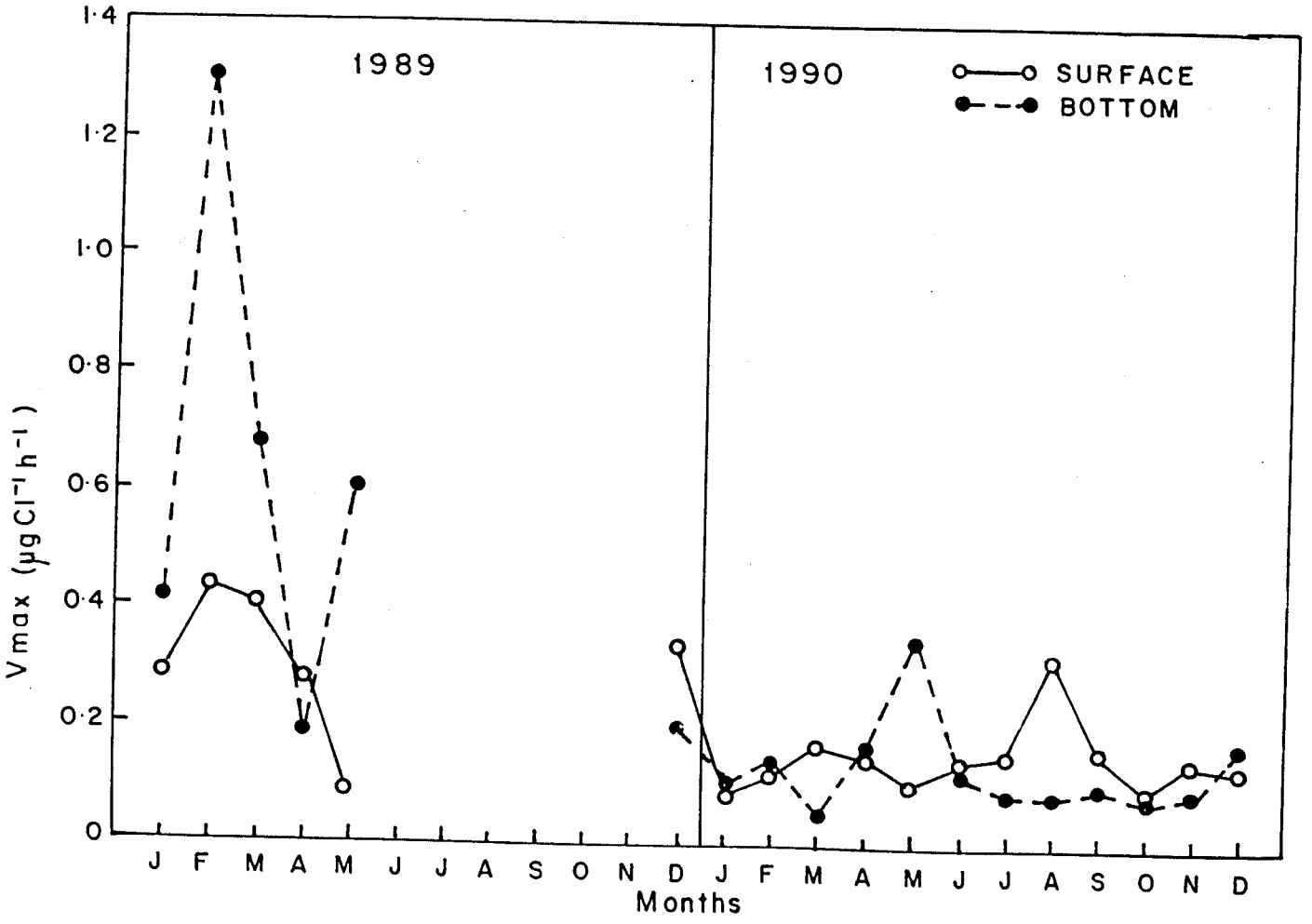

Fig. 6. Monthly variations in the maximum velocity of uptake of glucose in surface and bottom water at seasonal pond, Narakkal, 1989 and 1990.

Like perennial pond no significant variation was noticed over different seasons for surface and bottom water (Table 18 C & D), and between surface and bottom water for V_{max} of glucose (Table 30 E) ($P > 0.05$).

(c) $K_t + S_n$ of glucose ($\mu gC\ l^{-1}$)

(i) Perennial Pond

The results of figure 7 shows that in surface water, $K_t + S_n$ varied greatly from 1.425 $\mu gC\ l^{-1}$ to 7.825 $\mu gC\ l^{-1}$ during 1989 and in 1990 it ranged between 0.25 $\mu gC\ l^{-1}$ and 6.0 $\mu gC\ l^{-1}$. The highest value was recorded in April 1989 (7.825 $\mu gC\ l^{-1}$) and a decrease in values were observed in May and June, again in peak monsoon month July a slight increase in value (4.750 $\mu gC\ l^{-1}$) was recorded. From August onwards values fluctuated till the end of the year. During 1990, the highest peak value was encountered in August and the secondary peak was value recorded in October (4.475 $\mu gC\ l^{-1}$) and 3.95 $\mu gC\ l^{-1}$ being the teritary peak in March.

In bottom water two peak values were recorded, the highest being 7.72 $\mu gC\ l^{-1}$ in the month of August and the secondary peak value was noticed in March 1989 (6.975 $\mu gC\ l^{-1}$). There was only slight variation in the values during April to July and again from September 1989 onwards upto August 1990. The trend however, was for high value during September 1990, the value being 3.6 $\mu gC\ l^{-1}$ and the lowest was 0.475 $\mu gC\ l^{-1}$ in February 1990.

The seasonal mean was found to be minimum (2.80 ± 1.54) during post-monsoon and maximum (3.47 ± 2.55) was in pre-monsoon in surface

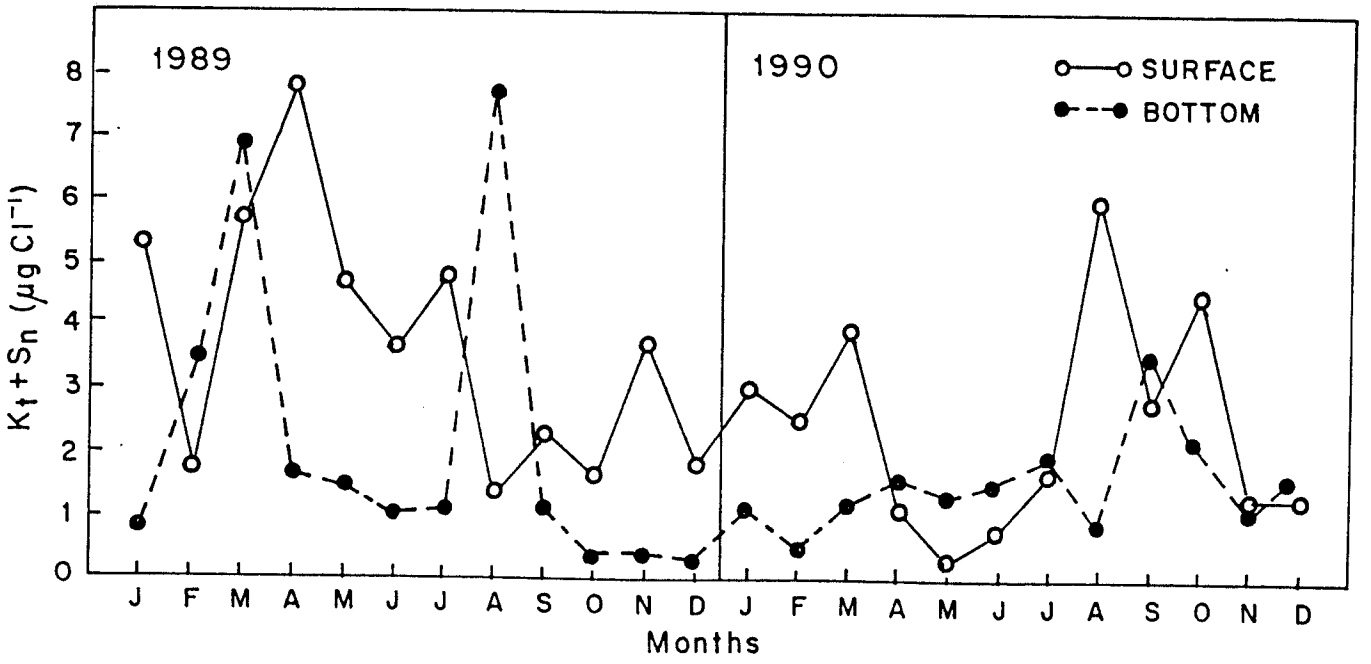

Fig. 7. Monthly variations in the sum of transport constant (K_t) and the natural substrate concentration (S_n) of glucose in surface and bottom water at perennial pond, Narakkal, 1989 and 1990.

water whereas in bottom water the maximum (2.37 ± 2.32) seasonal mean was obtained in monsoon and minimum (0.97 ± 0.65) during post-monsoon.

ANOVA over different seasons for surface and bottom water (Table 19 A & B) shows no significant variation ($P > 0.05$). ANOVA between surface and bottom water, (Table 30C) indicates significant variation for $K_t + S_n$ of glucose ($P < 0.05$). $K_t + S_n$ of glucose concentration was found higher in bottom water.

(ii) Seasonal Pond

The seasonal pond, the $K_t + S_n$ of glucose was ranged from 0.825 $\mu gC\ l^{-1}$ to 4.200 $\mu gC\ l^{-1}$, the highest value was recorded in April (4.200 $\mu gC\ l^{-1}$) and the lowest (0.825 $\mu gC\ l^{-1}$) was in February (Fig.8). The $K_t + S_n$ was varied slightly in January, March and May during 1989.

In 1990, the $K_t + S_n$ of glucose ranged between 0.875 $\mu gC\ l^{-1}$ in October, to 2.65 $\mu gC\ l^{-1}$ in August in surface water. Whereas in bottom water during 1989 two peak values were recorded in May and February, the value being 4.125 $\mu gC\ l^{-1}$ and 5.3 $\mu gC\ l^{-1}$ respectively. Lowest value (0.65 $\mu gC\ l^{-1}$) was recorded in December 1989. In 1990, the $K_t + S_n$ of glucose ranged between 0.175 $\mu gC\ l^{-1}$ in March 3.075 $\mu gC\ l^{-1}$ during May. Only slight variation was observed during June to November. Two intermediate peaks values for $K_t + S_n$ were recorded in February (2.075 $\mu gC\ l^{-1}$) and December (2.0 $\mu gC\ l^{-1}$) during 1990.

The seasonal mean in surface water of seasonal pond was maximum (2.068 ± 0.3986) in monsoon and minimum was obtained (1.025 ± 0.1322)

Table 19. One-way ANOVA for the sum of the transport 'Constant' and the natural substrate concentration of glucose in surface and bottom water at perennial and seasonal ponds.

	SOURCE	DF	SS	MSS	F	REMARKS
	PERENNIAL POND					
A.	Seasons	2	2.056	1.028	0.26	NS
	Error	21	84.322	4.015		
	Total	23	86.378			
B.	Seasons	2	9.354	4.677	1.35	NS
	Error	21	72.673	3.461		
	Total	23	82.026			
	SEASONAL POND					
C.	Seasons	2	0.323	0.162	0.28	NS
	Error	15	8.675	0.578		
	Total	17	8.999			
D.	Seasons	2	7.864	3.932	3.09	NS
	Error	15	19.087	1.272		
	Total	17	26.952			

A & C - Surface Water, B & D - Bottom Water

NS - Not Significant

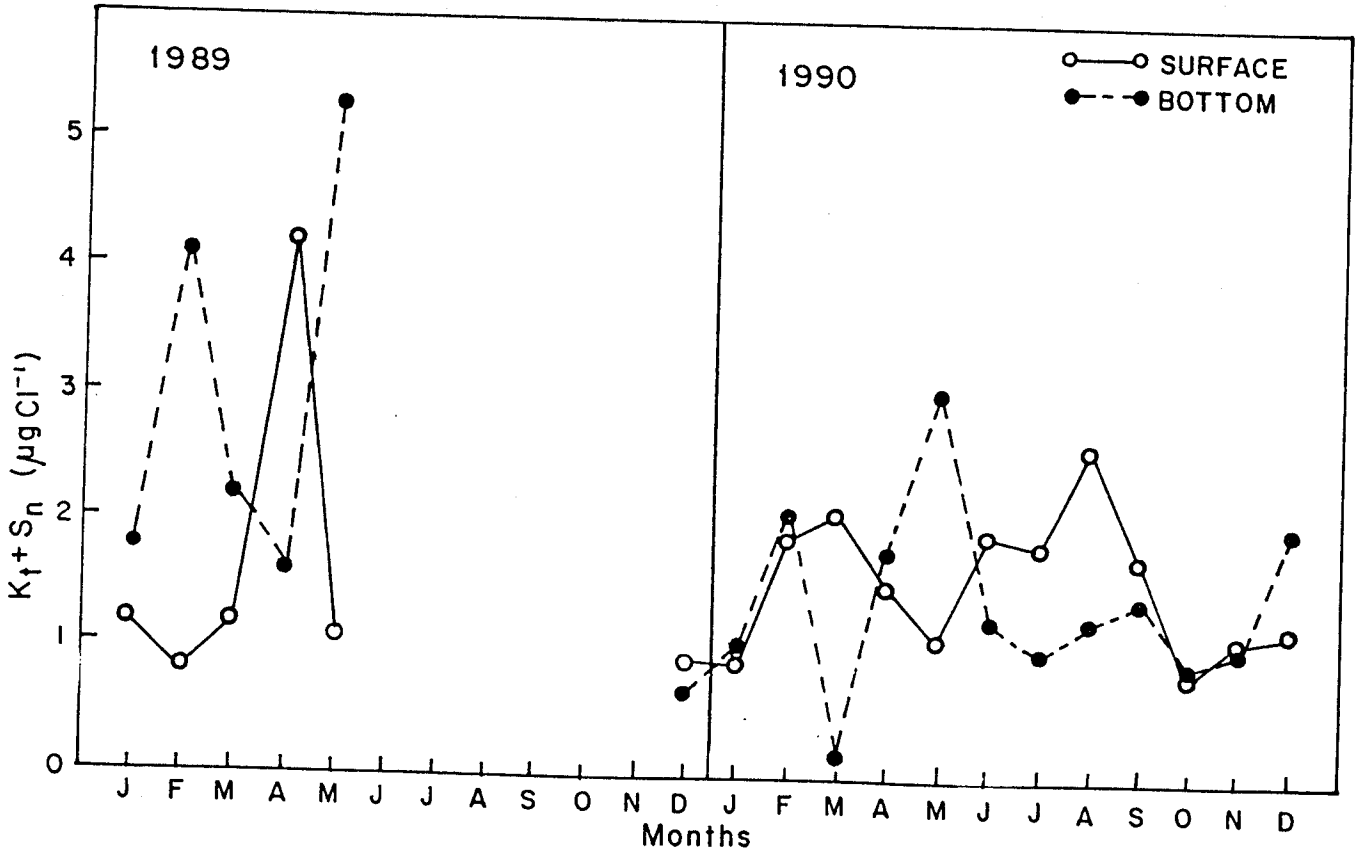

Fig. 8. Monthly variations in the sum of the transport constant (K_t) and the natural substrate concentration (S_n) of glucose in surface and bottom water at seasonal pond, Narakkal, 1989 and 1990.

Table 30. One-way ANOVA for the Kinetic parameters of glucose substrate in the perennial and seasonal ponds between surface and bottom water.

	SOURCE	DF	SS	MS	F	REMARKS
	PERENNIAL POND					
A.	Space	1	1.040	1.040	0.07	NS
	Error	46	710.169	15.438		
	Total	47	711.209			
B.	Space	1	0.418	0.418	5.44	SIG
	Error	46	3.536	0.077		(5%)
	Total	47	3.954			
C.	Space	1	17.690	17.690	4.83	SIG
	Error	46	168.405	3.661		(5%)
	Total	47	186.095			
	SEASONAL POND					
D.	Space	1	3.688	3.688	0.25	NS
	Error	34	492.313	14.480		
	Total	35	496.002			
E.	Space	1	0.055	0.055	0.91	NS
	Error	34	2.032	0.060		
	Total	35	2.087			
F.	Space	1	0.456	0.456	0.02	NS
	Error	34	472.105	20.526		
	Total	35	472.561			

A & D - Turnover time, B & E - Maximum Velocity of uptake.

C & F - The sum of transport 'Constant' and natural substrate concentration.

NS - Not Significant

SIG - Significant

during post-monsoon, in bottom water the maximum was during pre-monsoon and minimum occured in post-monsoon (Table 3).

Like perennial pond no significant variation was noticed over different seasons for surface and bottom water (Table 19 C and D); and between surface and bottom water for the $K_t + S_n$ of glucose (Table 30 F) ($P > 0.05$).

II. Results of acetate uptake experiments

Acetate uptake experiments started only from February 1989 onwards in both the ponds and from June to November 1989 the data was not collected at seasonal pond due to paddy cultivation. The results of acetate uptake experiments are derived from linear regression equation (Fig.1) as previously for glucose. The results are presented in figures 9, 10, 11, 12, 13 and 14 (Table 4 & 5). The statistical validity of the regressions of acetate was often found less than glucose.

(a) Turnover time of acetate: T_t (hrs)

(i) Perennial Pond

Three peaks were observed in the turnover time of acetate (Fig.9), the highest being in July during 1989 and bimodal peak was observed during August and September 1990. The increase in acetate turnover was roughly twice as great in bottom water as at surface water. The turnover time ranged from 3.07 hrs to 15.58 hrs in June and July 1989 respectively. In March 1990 the lowest range was observed, the T_t being 3.374 hrs in surface water. 8.296 hrs was the lowest recorded T_t in bottom water during 1990.

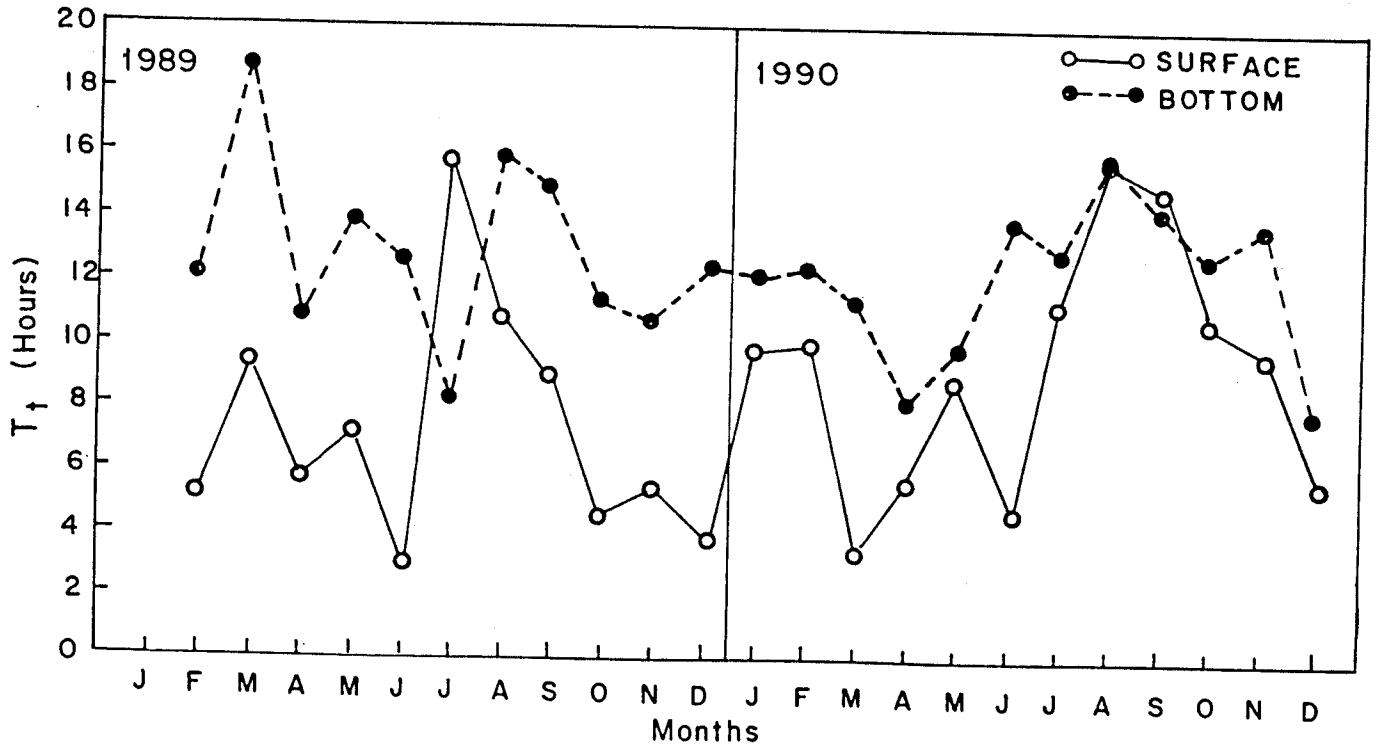

Fig. 9. Monthly variations in the turnover of sodium acetate in surface and bottom water of perennial pond, Narakkal, 1989 and 1990.

In surface water the lowest seasonal mean was recorded during pre-monsoon (6.95 ± 2.33). The highest mean value was obtained during monsoon (10.45 ± 4.74). The minimum seasonal mean 11.5 ± 1.89 was observed in post-monsoon and maximum (13.65 ± 2.48) was encountered during monsoon in bottom water (Table 4).

The results of ANOVA over different seasons for surface and bottom water (Table 20 A & B) reveals no significant variation ($P > 0.05$). ANOVA between surface and bottom water, (Table 31 A) indicates significant variation for T_t of sodium acetate ($P < 0.01$). T_t of acetate found to be higher in bottom water.

(ii) Seasonal Pond

Fig.10 illustrates the pattern of turnover time of acetate in surface water at seasonal pond. The turnover time fluctuated between 9.529 hrs in February 1989 and the highest was recorded as 17.38 hrs in December 1989. In 1990 the lowest range was recorded during November as 7.78 hrs and the highest 19.67 hrs in July in surface water.

In bottom water highest turnover time 18.72 hrs was recorded during December and the lowest 7.14 hrs in March 1989. 9.641 hrs was the lowest T_t in November and highest 18.32 hrs was observed in July 1990.

The minimum seasonal mean was obtained in post-monsoon (10.70 ± 3.92) and the maximum (17.31 ± 2.06) was encountered in monsoon months in surface water, whereas in bottom water the lowest seasonal mean was in pre-monsoon and highest mean was during monsoon season.

Table 4. Seasonal mean and standard deviation for kinetic parameters of sodium acetate in surface and bottom water at perennial pond.

Kinetic Parameters	Seasons		
	Pre-monsoon	Monsoon	Post-monsoon
PERENNIAL POND			
Surface Water			
T_t (hrs)	6.95 ± 2.33	10.45 ± 4.74	7.09 ± 2.86
V_{max} ($\mu gC\ l^{-1}h^{-1}$)	0.28 ± 0.07	0.24 ± 0.09	0.24 ± 0.06
K_t+S_n ($\mu gC\ l^{-1}$)	1.86 ± 0.81	2.93 ± 2.93	1.89 ± 1.14
Bottom Water			
T_t (hrs)	12.13 ± 3.13	13.65 ± 2.48	11.51 ± 1.89
V_{max} ($\mu gC\ l^{-1}h^{-1}$)	0.26 ± 0.06	0.25 ± 0.05	0.23 ± 0.06
$K_t + S_n$ ($\mu gC\ l^{-1}$)	3.25 ± 1.47	3.45 ± 0.98	3.01 ± 1.14

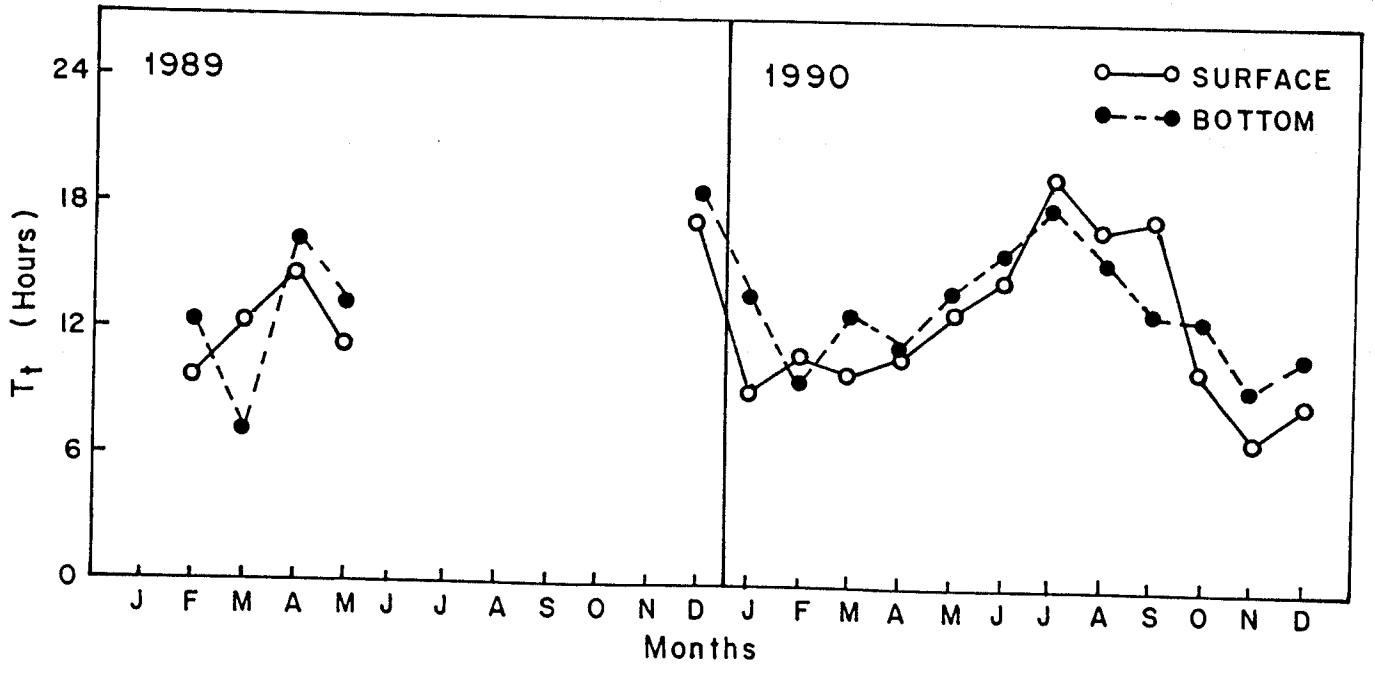

Fig. 10. Monthly variations in the turnover time of sodium acetate in surface and bottom water at seasonal pond, Narakkal, 1989 and 1990.

Table 20. One-way ANOVA for turn over time of sodium acetate in surface and bottom water at perennial and seasonal ponds during different seasons.

	SOURCE	DF	SS	MSS	F	REMARKS
	PERENNIAL POND					
A.	Seasons	2	61.325	30.663	2.51	NS
	Error	20	244.336	12.217		
	Total	22	305.662			
B.	Seasons	2	18.631	9.316	1.40	NS
	Error	20	133.408	6.670		
	Total	22	152.040			
	SEASONAL POND					
C.	Seasons	2	112.742	56.371	8.50	HI.SIG (1%)
	Error	14	92.813	6.629		
	Total	16	205.554			
D.	Seasons	2	34.912	17.456	2.15	NS
	Error	14	113.677	8.120		
	Total	16	148.588			

A & C - Surface Water, B & D - Bottom Water

NS - Not Significant

HI.SIG - Highly Significant

ANOVA (Table 20 C) shows that there is a significant variation between pre-monsoon and monsoon season (SE=1.577) and also between monsoon and post-monsoon (SE=1.727) in the turnover time of acetate at surface water ($P < 0.01$). For bottom water no significant variation was noticed over different seasons (Table 20 D); between surface and bottom water for T_t of acetate (Table 31 D) ($P > 0.05$).

(b) Maximum uptake velocity of acetate: V_{max} ($\mu gC\ l^{-1}h^{-1}$)

(i) Perennial Pond

The maximum velocity of uptake of acetate in surface water was highest during April and May, the value being 0.39 $\mu gC\ l^{-1}h^{-1}$ and 0.4 $\mu gC\ l^{-1}h^{-1}$ and the lowest 0.1128 $\mu gC\ l^{-1}h^{-1}$ was recorded in September 1989. In 1990 it ranged between 0.1885 $\mu gC\ l^{-1}h^{-1}$ in April and 0.445 $\mu gC\ l^{-1}h^{-1}$ in August.

In bottom water four minimum values were observed (Fig.11) the lowest 0.1278 $\mu gC\ l^{-1}h^{-1}$ occurred in November and highest T_t 0.3282 $\mu gC\ l^{-1}h^{-1}$ in March 1989. During 1990 the V_{max} values ranged between 0.2282 $\mu gC\ l^{-1}h^{-1}$ in February and 0.3664 $\mu gC\ l^{-1}h^{-1}$ in May.

Seasonal mean of V_{max} in surface and bottom water was minimum in post-monsoon and maximum during pre-monsoon (Table 4).

ANOVA over different seasons for surface bottom water (Table 21 A & B); between surface and bottom water, (Table 31 B) suggests no significant variation for the V_{max} of acetate ($P > 0.05$).

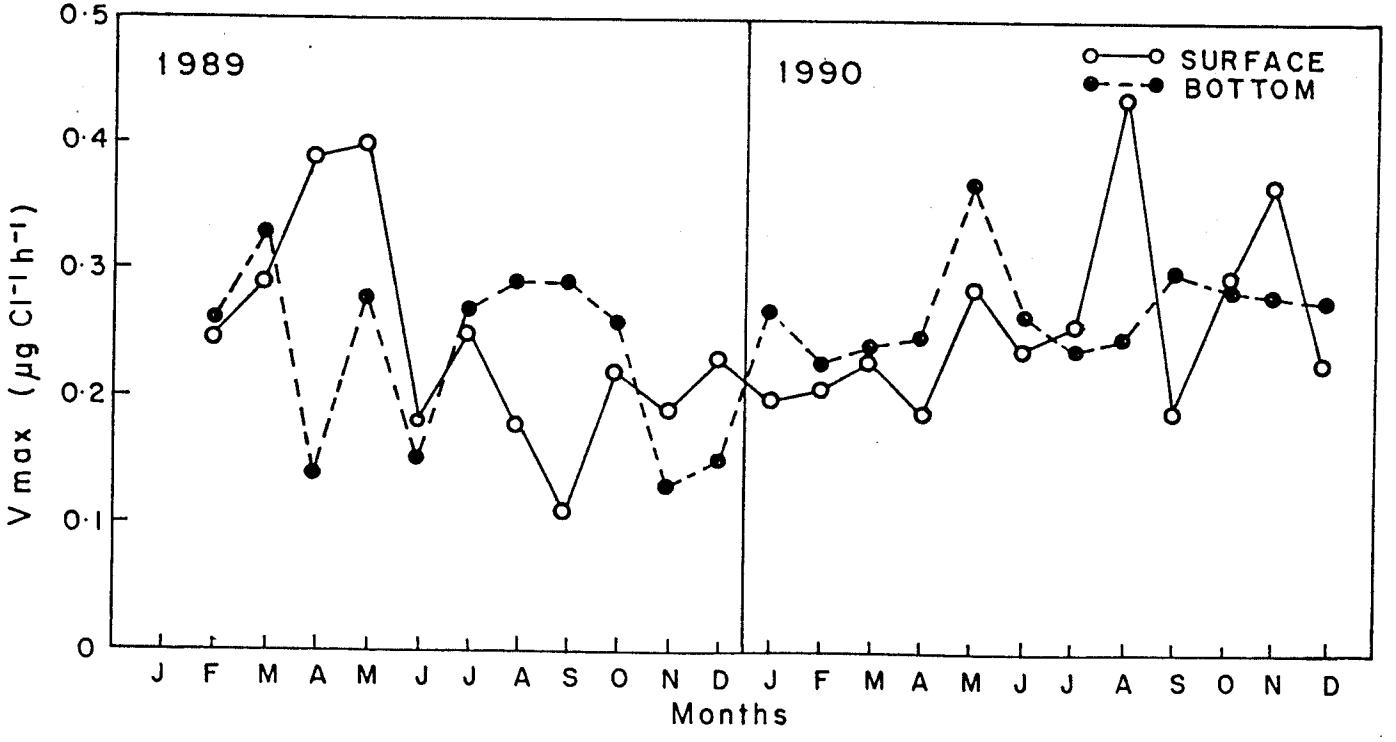

Fig. 11. Monthly variations in the maximum velocity of uptake of sodium acetate in surface and bottom water at perennial pond, Narakkal, 1989 and 1990.

Table 21. One-way ANOVA for maximum velocity of uptake of sodium acetate in surface and bottom water of perennial and seasonal ponds during different seasons.

	SOURCE	DF	SS	MSS	F	REMARKS
	PERENNIAL POND					
A.	Seasons	2	0.005	0.003	0.40	NS
	Error	20	0.137	0.007		
	Total	22	0.143			
B.	Seasons	2	0.003	0.001	0.37	NS
	Error	20	0.076	0.004		
	Total	22	0.079			
	SEASONAL POND					
C.	Seasons	2	0.009	0.004	1.32	NS
	Error	14	0.046	0.003		
	Total	16	0.054			
D.	Seasons	2	0.005	0.003	1.73	NS
	Error	14	0.021	0.002		
	Total	16	0.026			

A & C - Surface water, B & D - Bottom water

NS - Not Significant

(ii) Seasonal Pond

V_{max} of acetate in surface water at seasonal pond ranged from 0.1315 $\mu gC\ l^{-1}h^{-1}$ in April, 0.3537 $\mu gC\ l^{-1}h^{-1}$ in February during 1989 and in 1990 it ranged between 0.124 $\mu gC\ l^{-1}h^{-1}$ in February and 0.3182 $\mu gC\ l^{-1}h^{-1}$ in July, where as in bottom water in lowest range 0.1583 $\mu gC\ l^{-1}h^{-1}$ in May and highest was 0.2155 $\mu gC\ l^{-1}h^{-1}$ in February during 1989. In 1990 the recorded lowest V_{max} was 0.1872 $\mu gC\ l^{-1}h^{-1}$ in May and highest V_{max} of acetate were observed in January and December, the value being 0.2950 $\mu gC\ l^{-1}h^{-1}$ and 0.2803 $\mu gC\ l^{-1}h^{-1}$ respectively.

In surface water the maximum seasonal mean was obtained in pre-monsoon and the minimum during monsoon whereas in bottom water the maximum was in post-monsoon and minimum during pre-monsoon (Table 5).

Like perennial pond no significant variation was noticed over different seasons (Table 21 C & D); between surface and bottom water (Table 31 E) for V_{max} of acetate ($P > 0.05$).

(c) $K_t + S_n$ of Acetate (ugC l^{-1})

(i) Perennial Pond

The values for $K_t + S_n$ (Fig. 13) were characterised by large fluctuations throughout the study period. The $K_t + S_n$ of acetate ranged between 0.57 $\mu gC\ l^{-1}$ and 4.38 $\mu gC\ l^{-1}$ during 1989, the lowest $K_t + S_n$ was recorded in June and this was followed by a definite increase in July and suddenly decreased in September 1989 and the values were

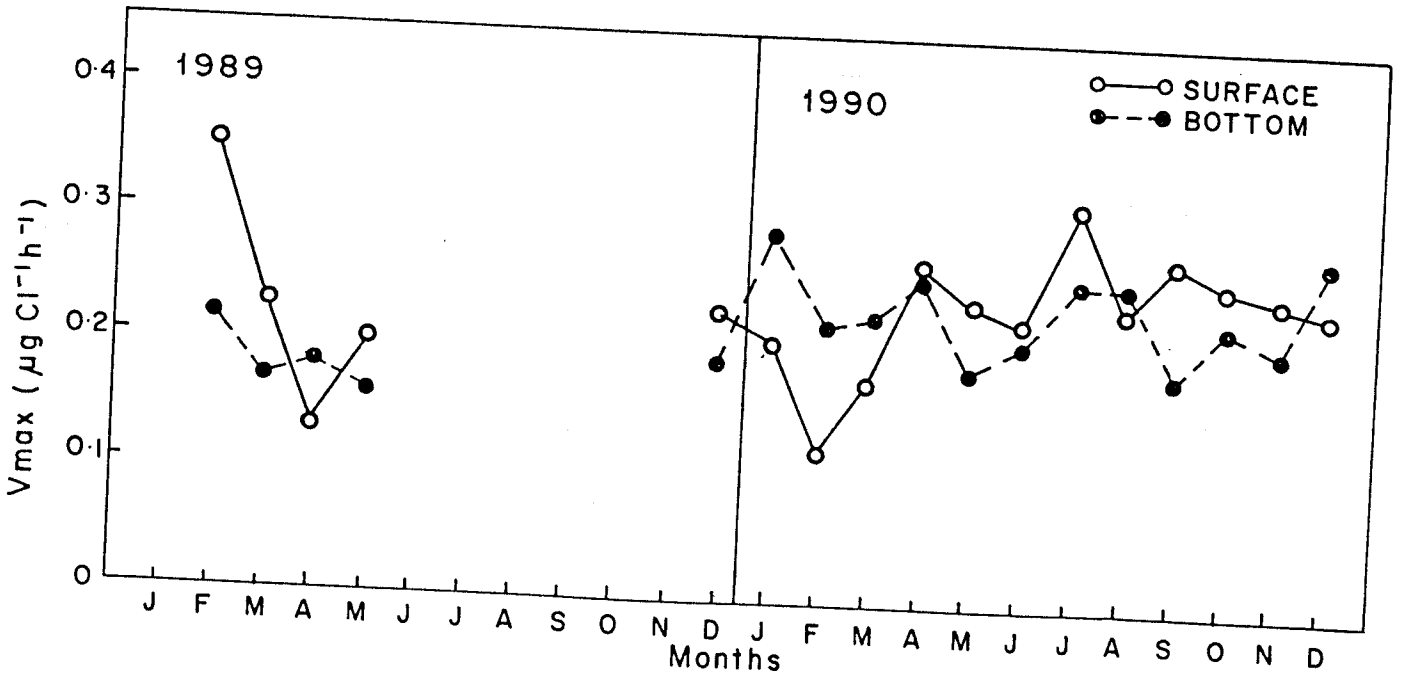

Fig. 12. Monthly variations in the maximum velocity of uptake of sodium acetate in surface and bottom water of seasonal pond, Narakkal, 1989 and 1990.

Table 5. Seasonal mean and standard deviation for kinetic parameters of sodium acetate at surface and bottom water in seasonal pond.

Kinetic Parameters	Seasons		
	Pre-monsoon	Monsoon	Post-monsoon
SEASONAL POND			
Surface Water			
T_t (hrs)	11.72 ± 1.63	17.31 ± 2.06	10.7 ± 3.92
V_{max} ($\mu gC\ l^{-1}h^{-1}$)	0.21 ± 0.07	0.27 ± 0.04	0.24 ± 0.02
K_t+S_n ($\mu gC\ l^{-1}$)	2.17 ± 0.57	3.9 ± 1.39	2.29 ± 0.8
Bottom Water			
T_t (hrs)	12.14 ± 2.76	15.75 ± 2.12	13.31 ± 3.41
V_{max} ($\mu gC\ l^{-1}h^{-1}$)	0.20 ± 0.04	0.23 ± 0.03	0.24 ± 0.05
K_t+S_n ($\mu gC\ l^{-1}$)	6.27 ± 2.23	4.71 ± 3.33	4.71 ± 2.73

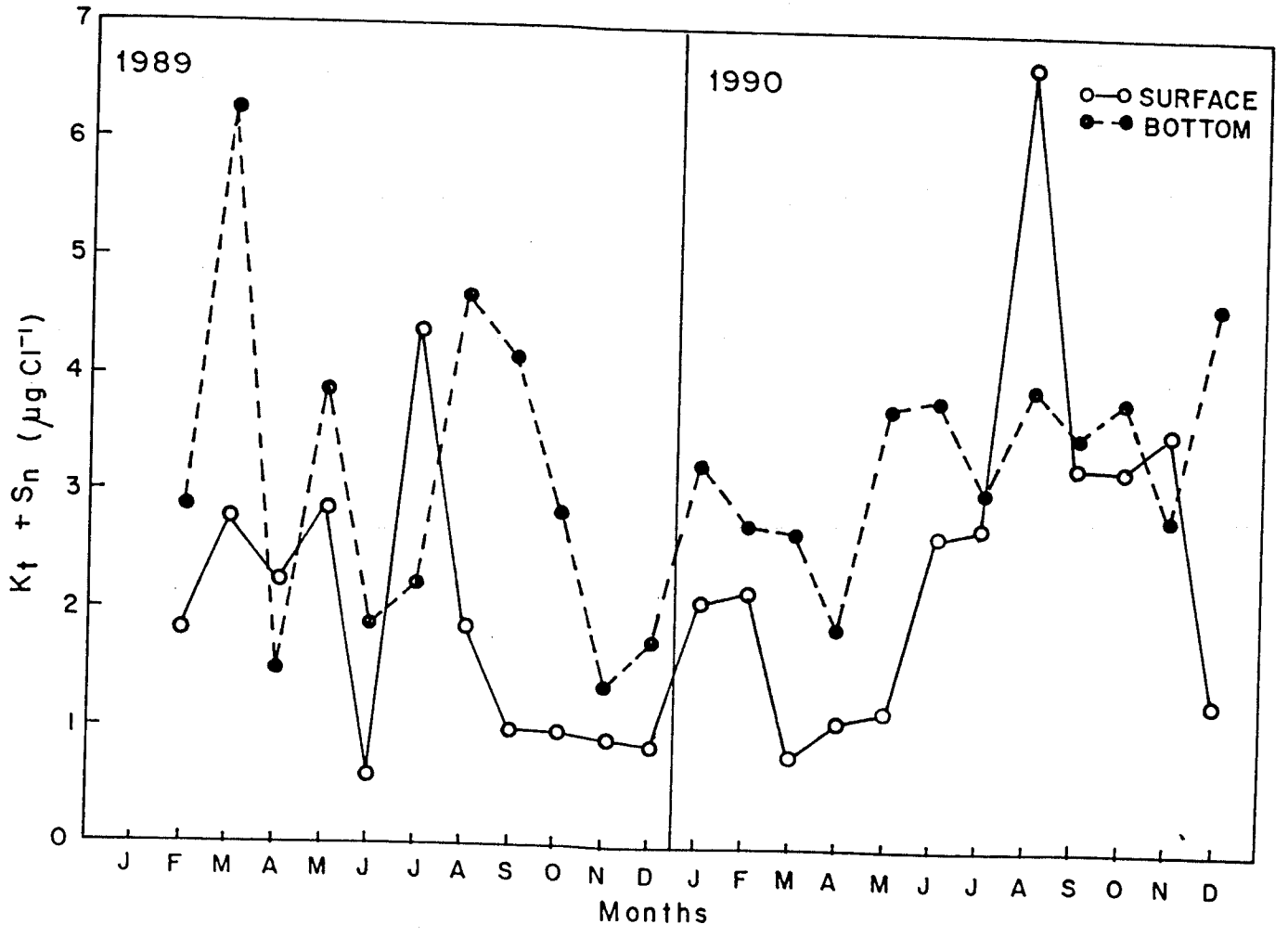

Fig. 13. Monthly variations in the sum of transport constant (K_t) and the natural substrate concentration (S_n) of sodium acetate in surface and bottom water at perennial pond, Narakkal, 1989 and 1990.

somewhat constant upto December 1989. In 1990 the lowest $K_t + S_n$ of acetate found to be 0.78 μgC l^{-1} in March and there was a definite increase 6.78 μgC l^{-1} in March and there was a definite increase 6.78 μgC l^{-1} during August.

Minimum seasonal mean in surface water was obtained 1.86 (± 0.81) during pre-monsoon and the maximum was 2.93 (± 1.77) in monsoon. In bottom water 3.01 (± 1.14) was the minimum and 3.45 (± 0.98) was recorded in monsoon (Table 4).

Analysis of variance over different seasons for surface and bottom water (Table 22 A & B) implies no significant variation ($P > 0.05$). ANOVA between surface and bottom water (Table 31 C) indicates significant variation for $K_t + S_n$ of acetate ($P < 0.05$). $K_t + S_n$ of acetate concentration was found high in bottom water.

(ii) Seasonal Pond

The values for $K_t + S_n$ (Fig. 14) were characterised by large variations. The $K_t + S_n$ of acetate in surface water during 1989 ranged from 1.8 μgC l^{-1} in April to 3.63 μgC l^{-1} in December. During 1990, $K_t + S_n$ ranged between 1.23 μgC l^{-1} in February and 5.85 μgC l^{-1} in February whereas in bottom water the highest $K_t + S_n$ of acetate concentration was 2.67 μgC l^{-1} in March 1989 and the lowest concentration 1.08 μgC l^{-1} was observed during December 1989 and in 1990, the range was higher being 1.83 μgC l^{-1} to 3.81 μgC l^{-1} in November and June respectively.

Table 22. One-way ANOVA for the sum of the transport 'Constant' and the natural substrate concentration of sodium-acetate in surface and bottom water of perennial and seasonal ponds during different seasons.

	SOURCE	DF	SS	MSS	F	REMARKS
	PERENNIAL POND					
A.	Seasons	2	5.849	2.925	1.48	NS
	Error	20	39.403	1.970		
	Total	22	45.252			
B.	Seasons	2	0.728	0.364	0.25	NS
	Error	20	29.701	1.485		
	Total	22	30.429			
	SEASONAL POND					
C.	Seasons	2	8.709	4.355	5.86	SIG (5%)
	Error	14	10.410	0.744		
	Total	16	19.119			
D.	Seasons	2	3.257	1.629	4.39	SIG (5%)
	Error	14	5.195	0.371		
	Total	16	8.452			

A & B-Surface water, B & D - Bottom water

NS - Not Significant

SIG - Significant

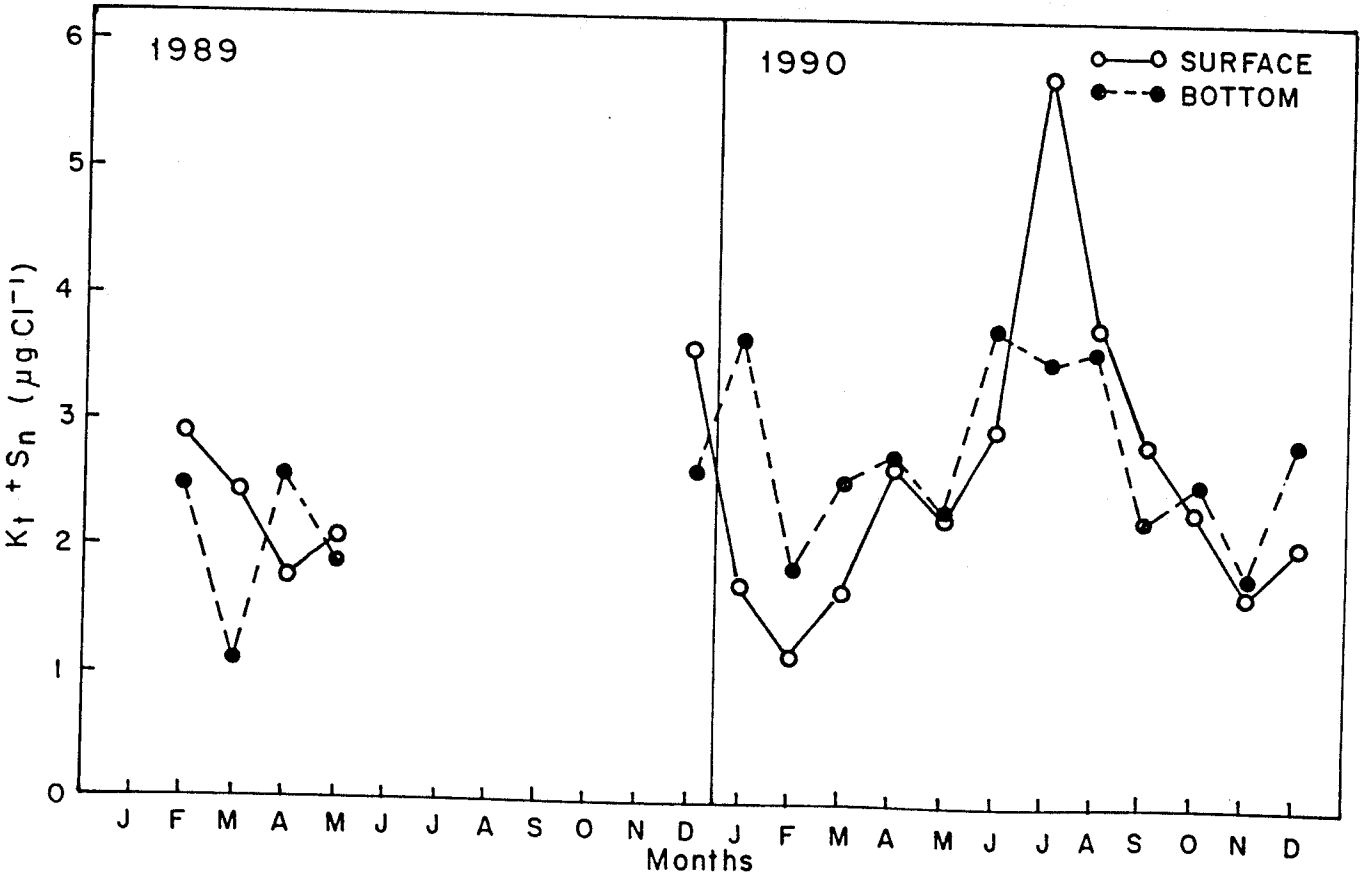

Fig. 14. Monthly variations in the sum of transport constant (K_t) and the natural substrate concentration (S_n) of sodium acetate in surface and bottom water at seasonal pond, Narakkal, 1989 and 1990.

Table 31. One-way ANOVA for the kinetic parameters of sodium acetate substrate in the perennial and seasonal ponds between surface and bottom water.

	SOURCE	DF	SS	MS	F	REMARKS
	PERENNIAL POND					
A.	Space	1	208.731	208.731	20.07	HI.SIG
	Error	44	457.702	10.402		(1%)
	Total	45	666.433			
B.	Space	1	0.000	0.000	0.03	NS
	Error	44	0.222	0.005		
	Total	45	0.222			
C.	Space	1	11.600	11.600	6.74	SIG
	Error	44	75.681	1.720		(5%)
	Total	45	87.282			
	SEASONAL POND					
D.	Space	1	3.094	3.094	0.28	NS
	Error	32	354.141	11.067		
	Total	33	357.235			
E.	Space	1	0.015	0.015	1.17	NS
	Error	32	0.403	0.013		
	Total	33	0.418			
F.	Space	1	0.005	0.005	0.01	NS
	Error	32	27.591	0.862		
	Total	33	27.596			

A & D - Turnover time, B & E - Maximum velocity of uptake

C & F - The sum of transport 'Constant' and the natural substrate concentration.

HI.SIG - Highly Significant

NS - Not Significant

SIG - Significant

Seasonal mean was minimum in surface water 2.17 ± 0.57 during pre-monsoon and maximum 3.90 ± 1.39 in monsoon. In bottom water the lowest seasonal mean 4.71 ± 2.73 was obtained in post-monsoon and 6.27 ± 2.23 was the highest recorded mean during pre-monsoon.

The results of ANOVA over different seasons for surface and bottom water (Table 22 C & D) specifies significant variation ($P < 0.05$) and in bottom water, there was a significant difference noticed between pre-monsoon and monsoon season (SE=0.373). ANOVA between surface and bottom water (Table 31 F) indicates no significant variation in the $K_t + S_n$ of acetate ($P > 0.05$).

PART - 2 (a) HYDROLOGICAL PARAMETERS

Monthly and seasonal mean variation in the hydrological parameters in the perennial pond such as temperature, pH, salinity and dissolved oxygen, during 1989 and 1990 are presented in Table 6 & 23; Fig. 15 & 17. Results of observation on the hydrological parameters in the (POKKALI FIELD) seasonal pond, which was limited to the pre-monsoon of 1989 are presented in Table 7 & 24; Fig. 16 & 18.

I. TEMPERATURE

(i) Perennial Pond

During 1989 the temperature values ranged between 28°C - 35°C. The values suddenly decreased in June to 28°C from 35°C recorded in May due to monsoon effect and reached the peak again (31°C) in October and declined later in November 1989 to January 1990 (Fig. 15), there

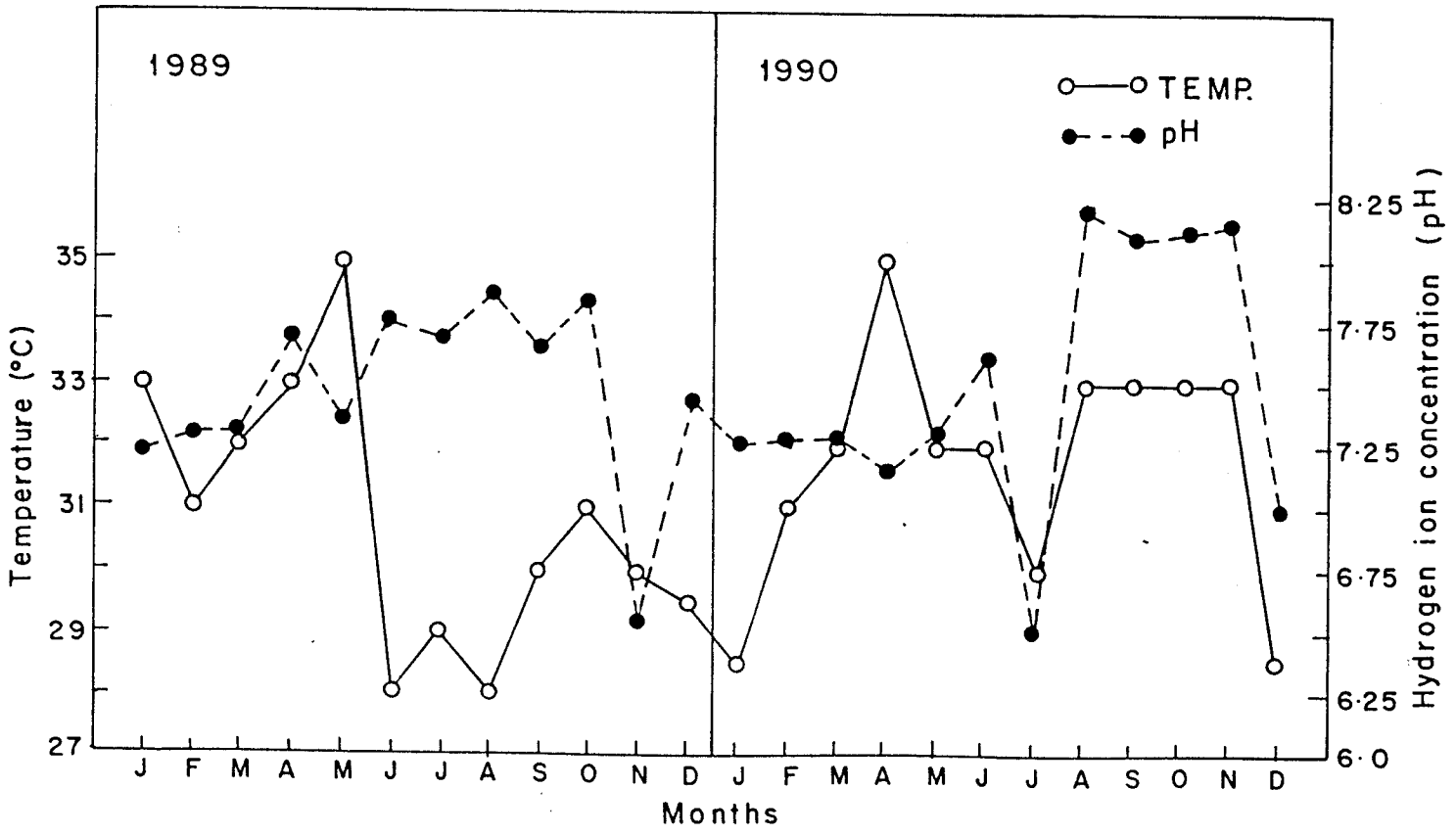

Fig. 15. Monthly variations of water temperature and pH in perennial pond at Narakkal during 1989 and 1990.

was a slight increase in February and March reaching the peak (35°C) in April and again it declined drastically in December 1990 (28.58). During pre-monsoon season of maximum solar radiation and warm weather the temperature throughout increased and recorded maximum in April 1990 with the onset of rains the water temperature reduced to 32°C in May and June and a clear thermal gradient develops in the water column. The thermal gradient persists until about August 1990. The temperature values were minimum during monsoon and the maximum occurred in pre-monsoon (Table 6).

Temperature relationship differed significantly (SE=0.946) between pre-monsoon and monsoon seasons (Table 23 A).

(ii) Seasonal Pond

Highest temperature (35°C) was recorded during April in both the years (Fig. 16) and the lowest was recorded in December in both the years. During 1989 the temperature ranged between 29 to 35°C and in 1990, the recorded range in temperature value was from 28 - 35½C. The maximum values were observed in pre-monsoon (Table 7) the value being 33 and the minimum was in post-monsoon 31 ± 2.34. Like perennial pond no significant variation was noticed over different seasons (Table 24 A) between water and sediment (Table 32 A) for temperaturee ($P > 0.05$).

II. HYDROGEN-ION-CONCENTRATION

(i) Perennial Pond

The pH showed seasonal fluctuation in both the years (Fig. 15). The values remained somewhat constant, the highest being 7.86 in August

Table 6. Seasonal mean and standard deviation for hydrological parameters in perennial pond.

Parameters	Seasons		
	Pre-monsoon	Monsoon	Post-monsoon
PERENNIAL POND			
Temperature (°C)	32.63 ± 1.59	30.38 ± 2.06	30.81 ± 1.98
pH	7.33 ± 0.15	7.67 ± 0.52	7.44 ± 0.56
Salinity (‰)	21.48 ± 2.30	4.39 ± 1.92	11.80 ± 4.99
Dissolved Oxygen (mg/l)	4.64 ± 0.81	5.48 ± 1.08	5.35 ± 0.66

Table 23. One-way ANOVA for hydrological parameters in perennial pond during different seasons.

	SOURCE	DF	SS	MSS	F	REMARKS
	PERENNIAL POND					
A.	Seasons	2	22.771	11.386	3.18	NS
	Error	21	75.219	3.582		
	Total	23	97.990			
B.	Seasons	2	1175.198	587.599	51.94	HI.SIG (1%)
	Error	21	237.562	11.312		
	Total	23	1412.760			
C.	Seasons	2	3.294	1.647	2.19	NS
	Error	21	15.808	0.753		
	Total	23	19.101			
D.	Seasons	2	0.484	0.242	1.21	NS
	Error	21	4.208	0.200		
	Total	23	4.692			

A - Temperature, B - Salinity, C - Dissolved oxygen, D - pH
NS - Not Significant
HI.SIG - Highly Significant

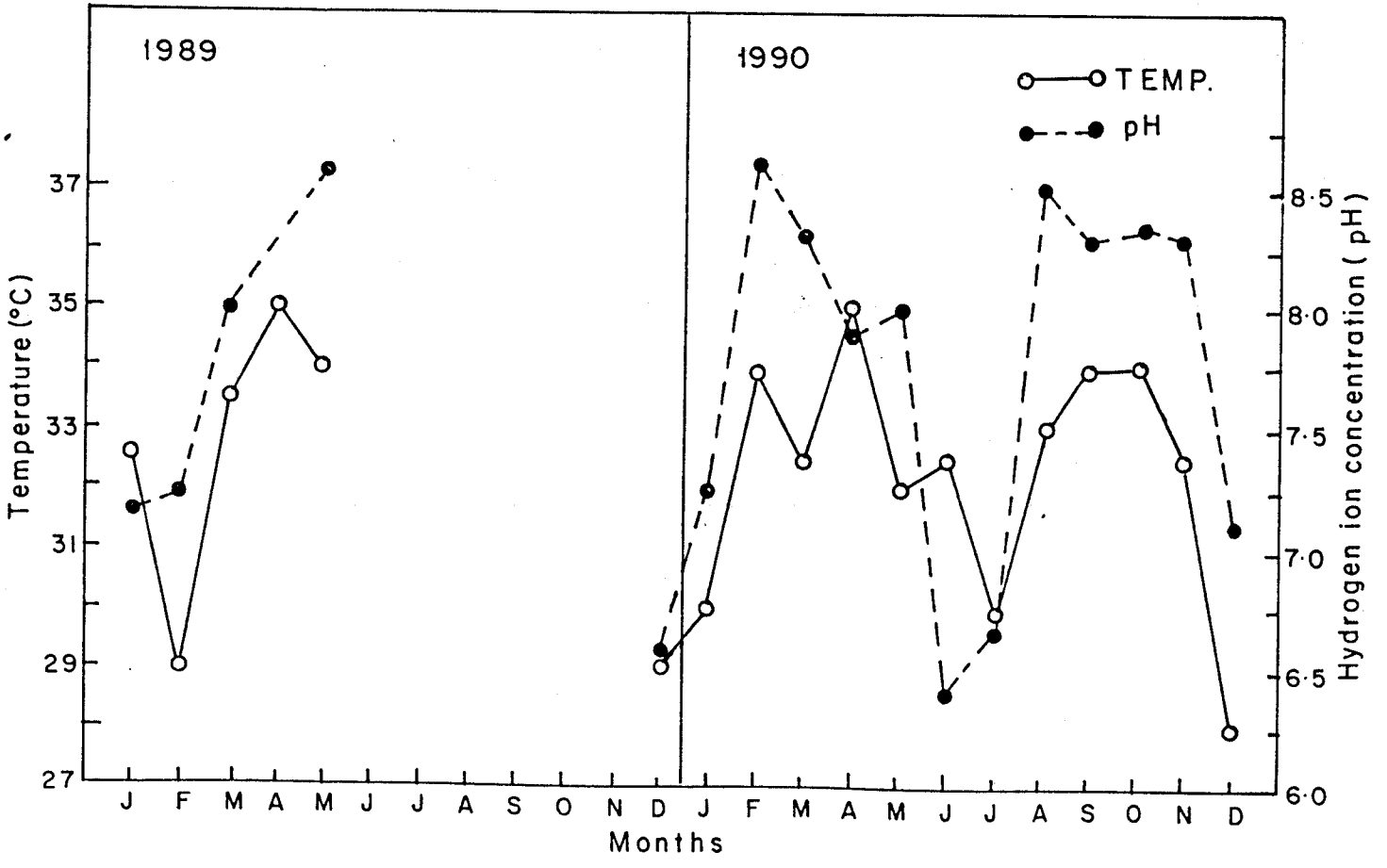

Fig. 16. Monthly variations of water temperature and pH in seasonal pond at Narakkal during 1989 and 1990.

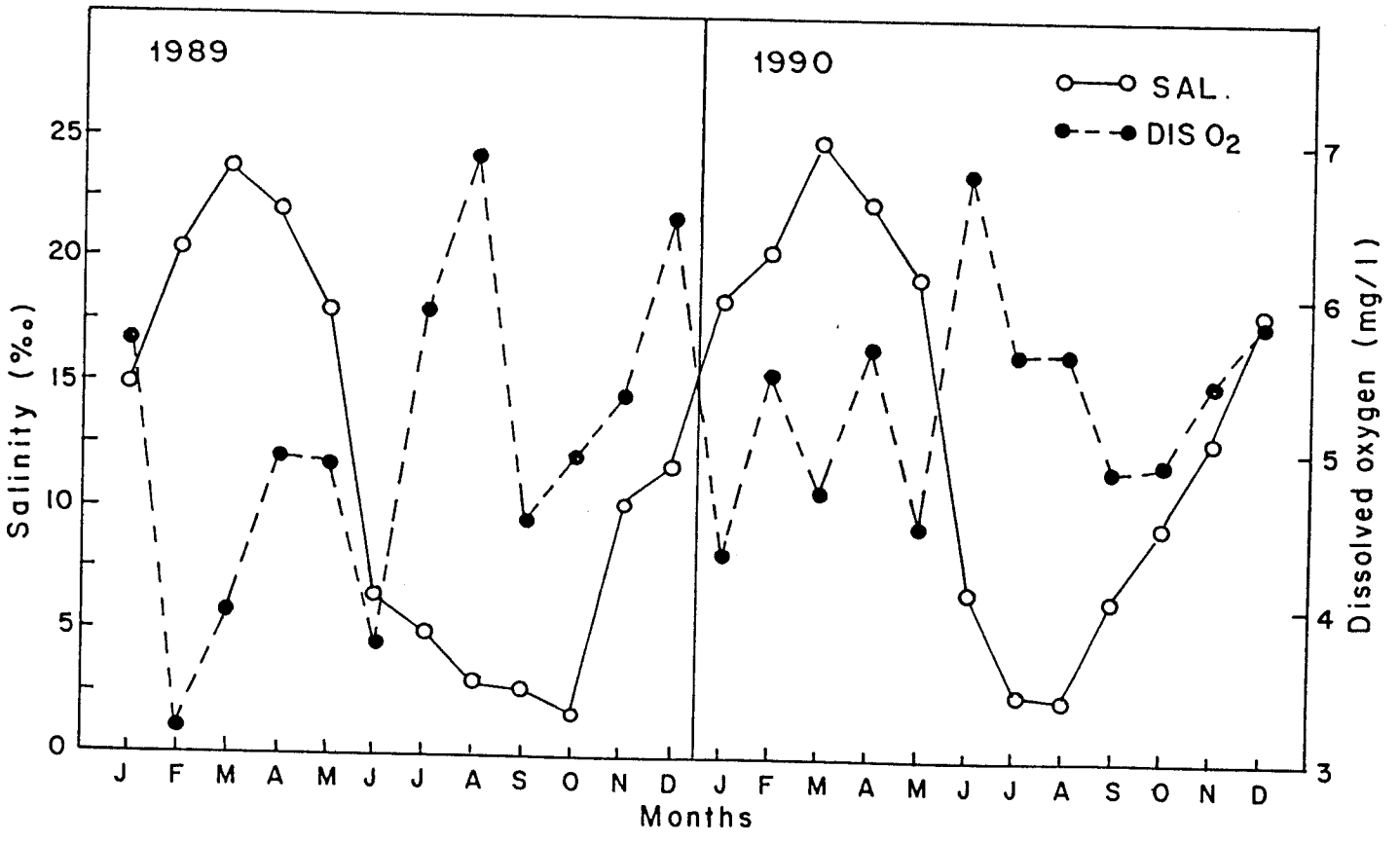

Fig. 17. Monthly variations of salinity and dissolved oxygen in perennial pond at Narakkal during 1989 and 1990.

1989 and the lowest pH 6.56 in November 1989. During 1990, the values remained high (in August to November 8.2, 8.1, 8.12 and 8.15) when condition are marine and fall progressively (in July the values being 6.5) as the system becomes freshwater dominated (Fig. 15). The seasonal mean was at its minimum during pre-monsoon and maximum was obtained in monsoon (Table 6).

The results of ANOVA over different seasons (Table 23 D); between water and sediment (Table 32 B) showed no significant variation in the hydrogen-ion-concentration ($P > 0.05$).

(ii) Seasonal Pond

The lowest pH (6.58) was recorded in December 1989 and 6.4 in June 1990, due to fresh water influence. The highest pH 8.57 was recorded in May 1989 and 8.6 in February 1990 (Fig. 16). During 1989 the range was between 7.22 to 8.57 only for pre-monsoon months and during 1990 it ranged from 6.4 to 8.6 respectively. Maximum seasonal mean was obtained in pre-monsoon and minimum was in post-monsoon months (Table 7).

The ANOVA suggests no significant variation in hydrogen ion-concentration over seasons and between water and sediment (Tables 24 D; 32 E) ($P > 0.05$).

III. SALINITY

(i) Perennial Pond

The salinity values ranged between 2.663-23.78 ppt in 1989 and 2.33-24.92 ppt in 1990. The peak value was obtained during pre-monsoon

Table 7. Seasonal mean and standard deviation for hydrological parameters in seasonal pond

Parameters	Seasons		
	Pre-monsoon	Monsoon	Post-monsoon
SEASONAL POND			
Temperature (°C)	33.13 ± 1.98	32.38 ± 1.70	31.0 ± 2.35
pH	8.12 ± 0.45	7.46 ± 1.09	7.45 ± 0.71
Salinity (‰)	19.54 ± 5.94	3.51 ± 2.75	14.39 ± 4.51
Dissolved Oxygen (mg/l)	7.11 ± 2.04	5.68 ± 0.49	4.51 ± 0.76

Table 24. One-way ANOVA for hydrological parameters in seasonal pond during different seasons.

	SOURCE	DF	SS	MSS	F	REMARKS
	SEASONAL POND					
A.	Seasons	2	15.563	7.781	1.84	NS
	Error	15	63.563	4.238		
	Total	17	79.125			
B.	Seasons	2	685.018	342.509	14.36	HI.SIG (1%)
	Error	15	357.800	23.853		
	Total	17	1042.818			
C.	Seasons	2	23.472	11.737	5.38	SIG (5%)
	Error	15	32.740	2.183		
	Total	17	56.211			
D.	Seasons	2	1.976	0.988	1.98	NS
	Error	15	7.498	0.500		
	Total	17	9.474			

A - Temperature, B - Salinity, C - Dissolved Oxygen, D - pH

NS - Not Significant

HI.SIG - Highly Significant

SIG - Significant

season. The values gradually increased from January and after reaching the peak in March in both the years, subsequently the values have declined to 2.663 ppt in 1989 and 2.33 ppt in 1990 due to the influx of fresh water during the monsoon season (Fig.17).

Seasonal mean was lowest in monsoon 4.39 (± 1.92) and in pre-monsoon the maximum mean was obtained the value being 21.48 (± 2.30), (Table 6). ANOVA for salinity (Table 23 B) shows that there is a significant differences between pre-monsoon and monsoon (SE=1.682), pre-monsoon and post-monsoon (SE=1.682), monsoon and post-monsoon (SE=1.682) ($P < 0.01$).

(ii) Seasonal Pond

The salinity has ranged between 9.985-23.39 ppt during 1989. The lowest peak was in May 1989 and the highest peak was recorded in April for both the years (Fig.18). During 1990, the salinity ranged from 1.79 to 25.9 ppt, the lowest value was recorded in August (1.79 ppt).

Highest seasonal mean recorded in pre-monsoon (Table 7) and in monsoon the seasonal mean was found to be minimum. Like perennial pond (Table 24 B) there is a significant differences noticed between pre-monsoon and monsoon (SE=2.991), monsoon and post-monsoon (SE=3.153) ($P < 0.01$).

IV. DISSOLVED OXYGEN

(i) Perennial Pond

The dissolved oxygen values ranged between 3.136-6.86 mg/l in 1989 (Fig.18). There was a gradual increase in oxygen values from February

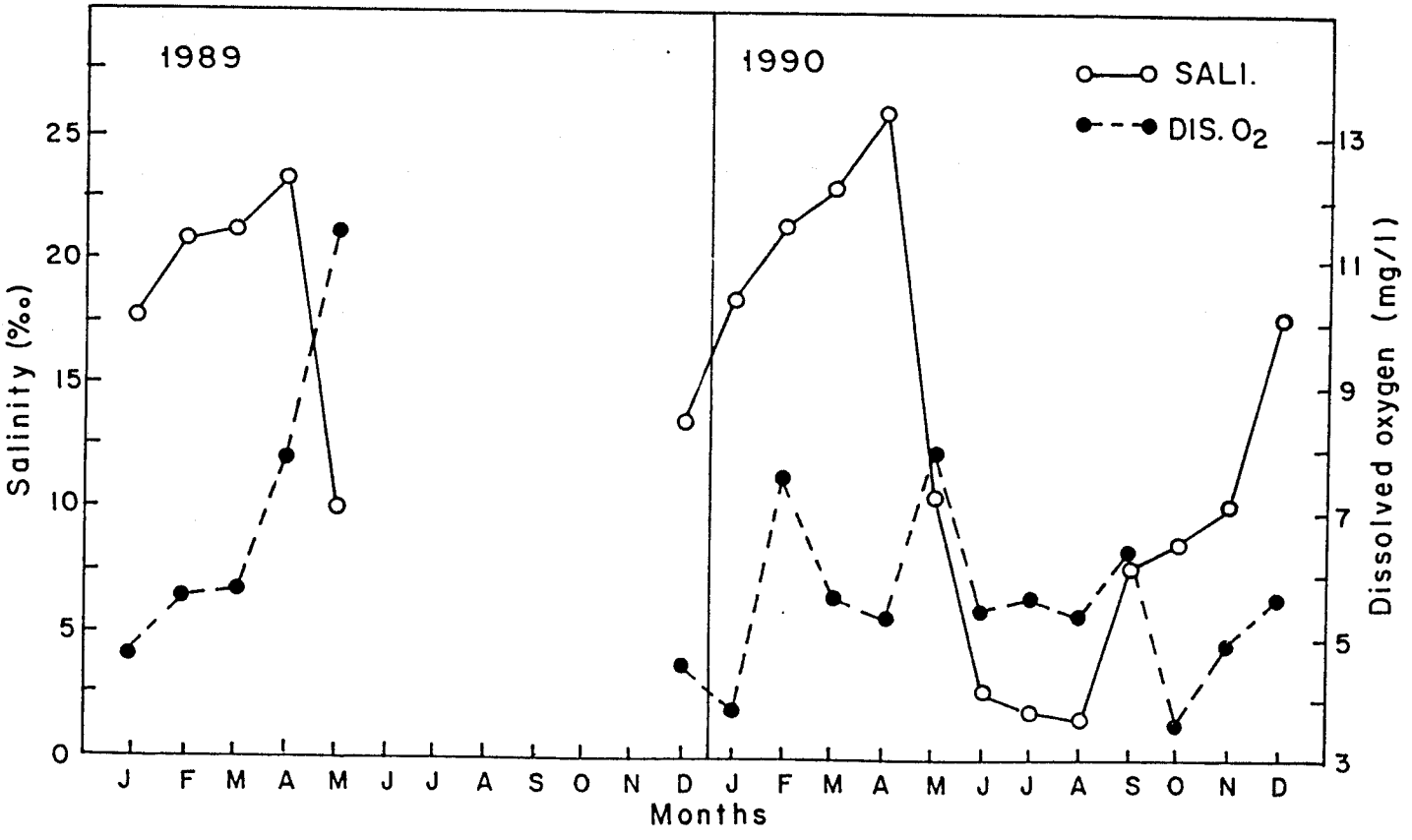

Fig. 18. Monthly variations of salinity and dissolved oxygen in seasonal pond at Narakkal during 1989 and 1990.

1989 onwards upto May 1989 and declines in June and again increased suddenly in July, August and declined sharply in September and gradual increase was recorded till December 1989. In 1990 highest peak was observed in June (6.768 mg/l) and it gradually declined till October and again increased in November and December. Seasonal mean was minimum in pre-monsoon 4.64 (± 0.81) and the maximum was obtained in monsoon months (5.48 ± 1.08) (Table 6). The results of ANOVA (Table 23 C) for dissolved oxygen implies that there is no significant variation over seasons ($P > 0.05$).

(ii) Seasonal Pond

The dissolved oxygen values was found to be higher (11.368 mg/l) in May 1989 (Fig.18) and lower in December 1989. The values being 4.508-11.368 mg/l. The peak value was recorded (7.898 mg/l) in May and minimum 3.57 mg/l in October 1990. Seasonal mean was maximum in pre-monsoon 7.11 (± 0.24) and minimum in post-monsoon (4.51 ± 0.76) (Table 7). ANOVA (Table 24 C) for dissolved oxygen indicates that there is a significant differences between pre-monsoon and post-monsoon (SE=0.798) ($P < 0.05$).

PART - 2 (b) SEDIMENT PARAMETERS

I. TEMPERATURE

(i) Perennial Pond

During 1989 the sediment temperature ranged between 28-34°C, with high values recorded during the pre-monsoon season (Fig. 19). The

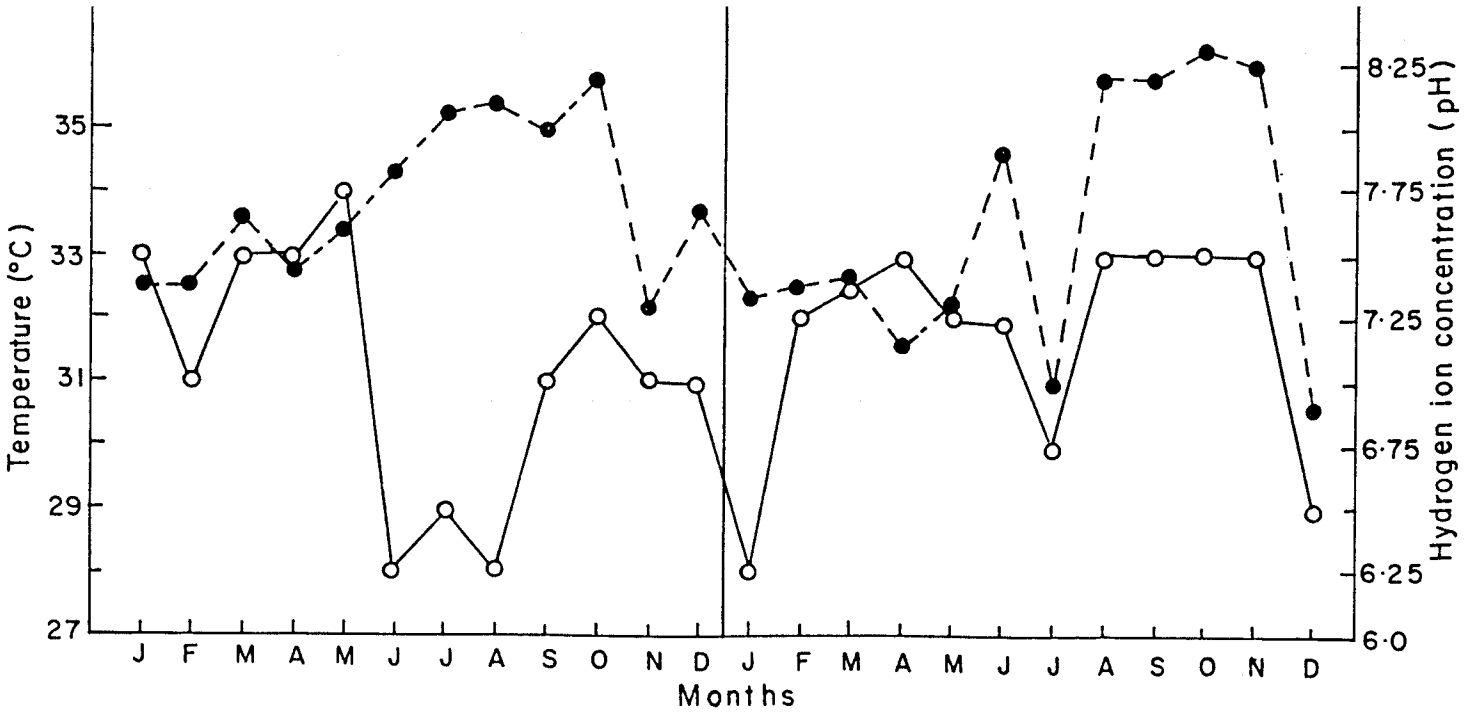

Fig. 19. Monthly variations of sediment temperature and pH in perennial pond at Narakkal during 1989 and 1990.

values gradually increased from January and after reaching the peak in May (34°C to 28°C) declined in the monsoon season, again during onset of monsoon it tended to show the increasing trend. During 1990, the recorded range in temperature was between 28 and 33°C. During pre-monsoon the increasing trend occurred in February to April and during monsoon gradual decline was noted and again in August to November the temperature values were constant (33°C) and again in December it decreased to 29°C. The obtained seasonal mean was maximum during pre-monsoon and minimum was in monsoon (Table 8).

ANOVA (Table 25 A) showed that no significant variation was found over seasons for sediment temperature ($P > 0.05$).

(ii) Seasonal Pond

During 1989 the temperature ranged between 29.5 to 34°C, during 1990, the recorded range was between 29.5 to 35°C. However the temperature values evinced variation when compared to the previous year. Peak value of temperature was recorded in April for both the year in pre-monsoon (Fig. 20). During 1990 secondary peaks were recorded in August and September (33.5°C) which gradually showed reducing tendency. The seasonal mean was maximum during pre-monsoon season and minimum was in post-monsoon (Table 8).

Like perennial pond no significant variation was noticed over seasons for sediment temperature (Table 25 C) ($P > 0.05$).

Table 8. Seasonal mean for sediment temperature and pH in perennial and seasonal ponds.

Parameters	Seasons		
	Pre-monsoon	Monsoon	Post-monsoon
PERENNIAL POND			
Sediment			
Temperature (°C)	32.56 ± 0.90	30.56 ± 2.16	31.18 ± 1.85
pH	7.41 ± 0.16	7.91 ± 0.39	7.66 ± 0.53
SEASONAL POND			
Sediment			
Temperature	32.75 ± 1.69	32.37 ± 1.65	30.92 ± 1.24
pH	7.28 ± 0.31	7.6 ± 0.87	7.97 ± 0.97

Table 25. One-way ANOVA for sediment temperature and pH in perennial and seasonal ponds during different seasons

	SOURCE	DF	SS	MSS	F	REMARKS
	PERENNIAL POND					
A.	Seasons	2	16.750	8.375	2.82	NS
	Error	21	62.406	2.972		
	Total	23	79.156			
B.	Seasons	2	0.965	0.483	3.19	NS
	Error	21	3.174	0.151		
	Total	23	4.140			
	SEASONAL POND					
C.	Seasons	2	12.049	6.024	2.52	NS
	Error	15	35.896	2.393		
	Total	17	47.945			
D.	Seasons	2	1.639	0.820	1.59	NS
	Error	15	7.728	0.515		
	Total	17	9.368			

A & C - Temperature, B & D - pH

NS - Not Significant

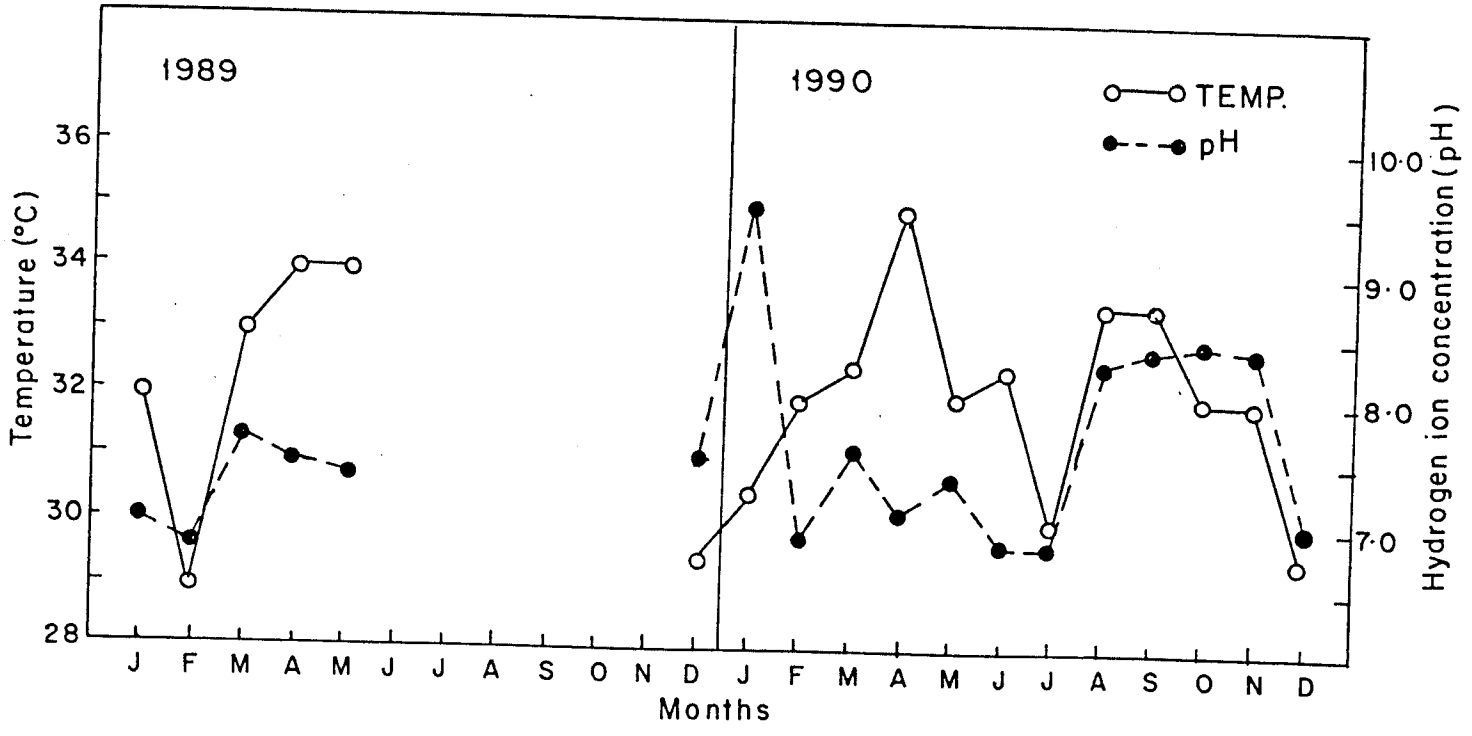

Fig. 20. Monthly variations of sediment temperature and pH in seasonal pond at Narakkal during 1989 and 1990.

II. HYDROGEN-ION-CONCENTRATION

(i) Perennial Pond

During 1989 the recorded value in pH was between 7.30 and 8.19. A gradual increasing trend occurred in pre-monsoon months and somewhat high concentrations were recorded during monsoon. The highest peak was observed in October (8.19) and the lowest pH was recorded in November (7.30) (Fig.19).

During 1990, the hydrogen-ion-concentration ranged between 6.9 to 8.3. A gradual increasing tendency occurred in January to March and again it declined in April and July. High concentrations were recorded in August and September being monsoon months and October and November being post-monsoon months. The lowest value was recorded in December (6.96). The seasonal mean was obtained maximum during monsoon and minimum was in pre-monsoon (Table 8).

ANOVA results for the hydrogen-ion-concentration(Table 25B) implies that there is no significant variation was found over seasons ($P > 0.05$).

(ii) Seasonal Pond

Hydrogen-ion-concentration in seasonal pond ranged between 6.8 to 7.63. The highest value was recorded in March 1989 and lowest was in February 1989. A decrease in values were noticed in pre-monsoon months during 1990, the values were ranged from 6.85 - 9.5. The highest peak occurred in January (9.5) and a sudden decline was recorded in

February and a gradual increase observed during April and May again the values decreased in June and July. After July increasing trend was noticed till November later it declines (7.0). Seasonal mean was obtained maximum in post-monsoon and minimum was in pre-monsoon (Table 8).

Like perennial pond no significant variation was noticed for hydrogen-ion-concentration over seasons (Table 25 D) ($P > 0.05$).

PART - 3 (a) PRIMARY PRODUCTIVITY

Results of observation on seasonal variation in the rate of primary productivity (gross and net production) during 1989 and 1990 are presented in Table 9 and Fig. 21 & 22.

(i) Perennial Pond

During 1989 the values of gross primary productivity ranged between 392 mgC/m^3/day in April and 1960 mgC/m^3/day in June. The primary productivity rate gradually decreased from January upto April and increased to the peak in June. After June only slight variations observed during post-monsoon months without any sharp peaks (Fig. 21).

During 1990, a decrease was observed in January (564 mgC/m^3/day). This is followed by an increase in February 1692 mgC/m^3/day decrease in March (940 mgC/m^3/day) and again sharply increased and attained highest value of the year in April the value being 2820 mgC/m^3/day. In May and June the values decreased gradually and the lowest recorded value of 564 mgC/m^3/day was in June. The secondary peak was observed

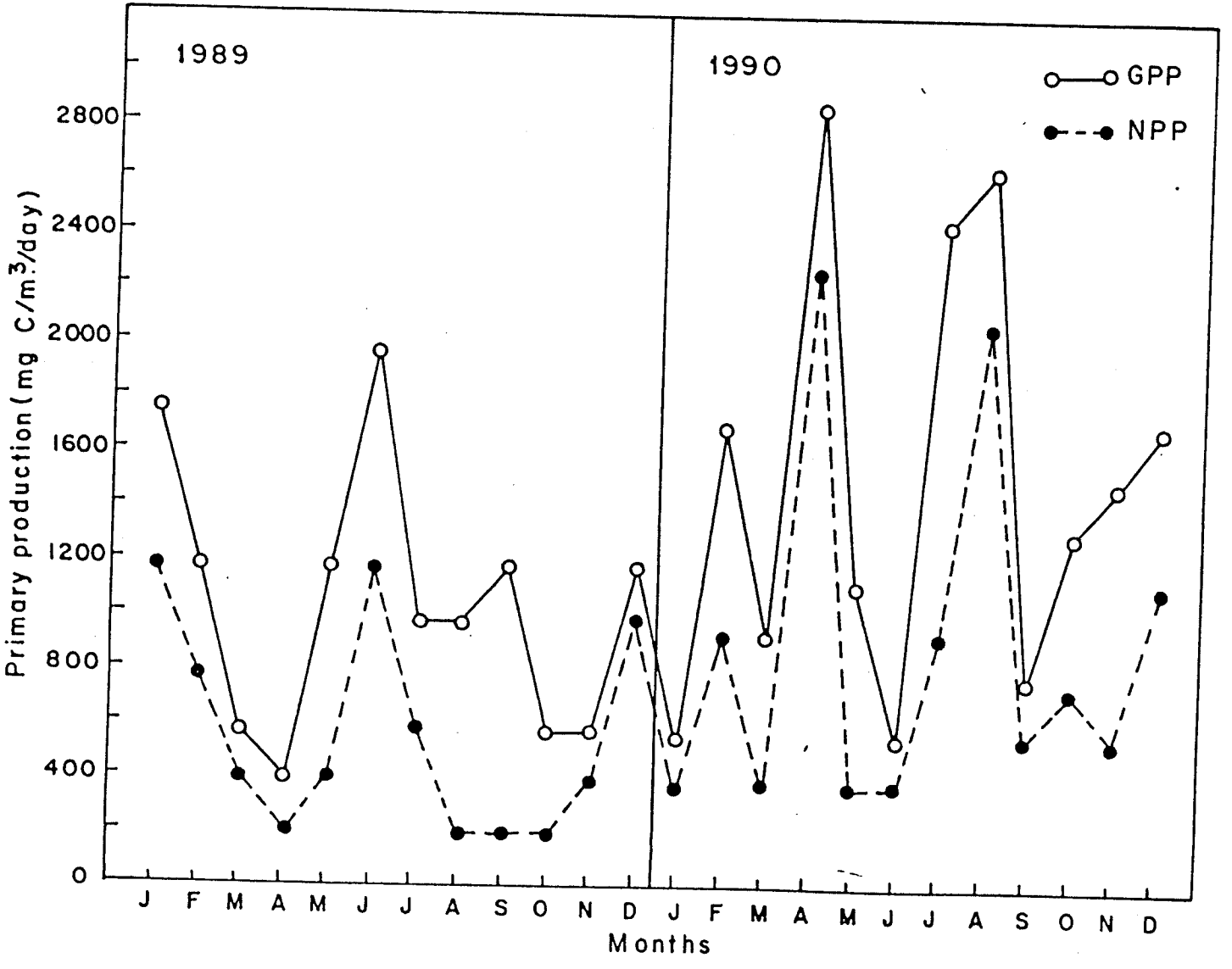

Fig. 21. Monthly variations in gross and net primary production in perennial pond at Narakkal during 1989 and 1990.

in July and tertiary was in August and declined sharply in September and gradually increased in the later part of the year.

Maximum seasonal mean was observed in monsoon and minimum mean was in post-monsoon (Table 9).

Net primary productivity ranged between 196 mgC/m^3/day to 1176 mgC/m^3/day during 1989 (Fig. 21). Two highest peaks were observed in January and June. One secondary peak was found in December. The values were somewhat constant from August to October.

The trend in net primary production was different during 1990, the highest being in April 2256 mgC/m^3/day. The secondary peak was observed in August the value being 2068 mgC/m^3/day. The lowest value 376 mgC/m^3/day was recorded during the months March, May and June.

Seasonal mean was maximum during monsoon 763 (± 628.82) and minimum was 694 (± 370.68) in post-monsoon months (Table 9).

Analysis of variance (Table 26 A & B) over different seasons for primary productivity (gross and net production) showed no significant variation ($P > 0.05$).

(ii) Seasonal Pond

Compared to perennial pond gross primary production value of seasonal pond were higher during the period of study. The highest value of 6468 mgC/m^3/day was recorded during April 1989 (Fig.22) the lowest 1372 mgC/m^3/day in March.

Table 9. Seasonal mean and standard deviation for primary production in perennial and seasonal ponds.

Parameters	Seasons		
	Pre-monsoon	Monsoon	Post-monsoon
PERENNIAL POND			
Gross Primary Production (mg C/m^3/day)	1239.00 ± 751.73	1436.00 ± 795.77	1149.00 ± 507.01
Net Primary Production (mg C/m^3/day)	714.00 ± 669.53	763.00 ± 628.82	694.00 ± 370.68
SEASONAL POND			
Gross Primary Production (mg C/m^3/day)	2837.00 ± 1697.76	2585.00 ± 1259.97	1564.66 ± 792.17
Net Primary Production (mg C/m^3/day)	1880.00 ± 1922.21	728.5 ± 310.57	1309.67 ± 884.36

Table 26. One-way ANOVA for gross and net primary production in perennial and seasonal ponds

	SOURCE	DF	SS	MSS	F	REMARKS
	PERENNIAL POND					
A.	Seasons	2	34472.	172370.	0.36	NS
	Error	21	10187792.	485132.		
	Total	23	10532532.			
B.	Seasons	2	20008.	1004.	0.03	NS
	Error	21	6869192.	327104.		
	Total	23	6889200.			
	SEASONAL POND					
C.	Seasons	2	5817888.	2908944.	1.55	NS
	Error	15	28077200.	1871813.375		
	Total	17	33895088.			
D.	Seasons	2	3675000.	1837500.	0.92	NS
	Error	15	30064088.	2004272.500		
	Total	17	33739088.			

A & C - Gross primary production

B & D - Net primary production

NS - Not Significant

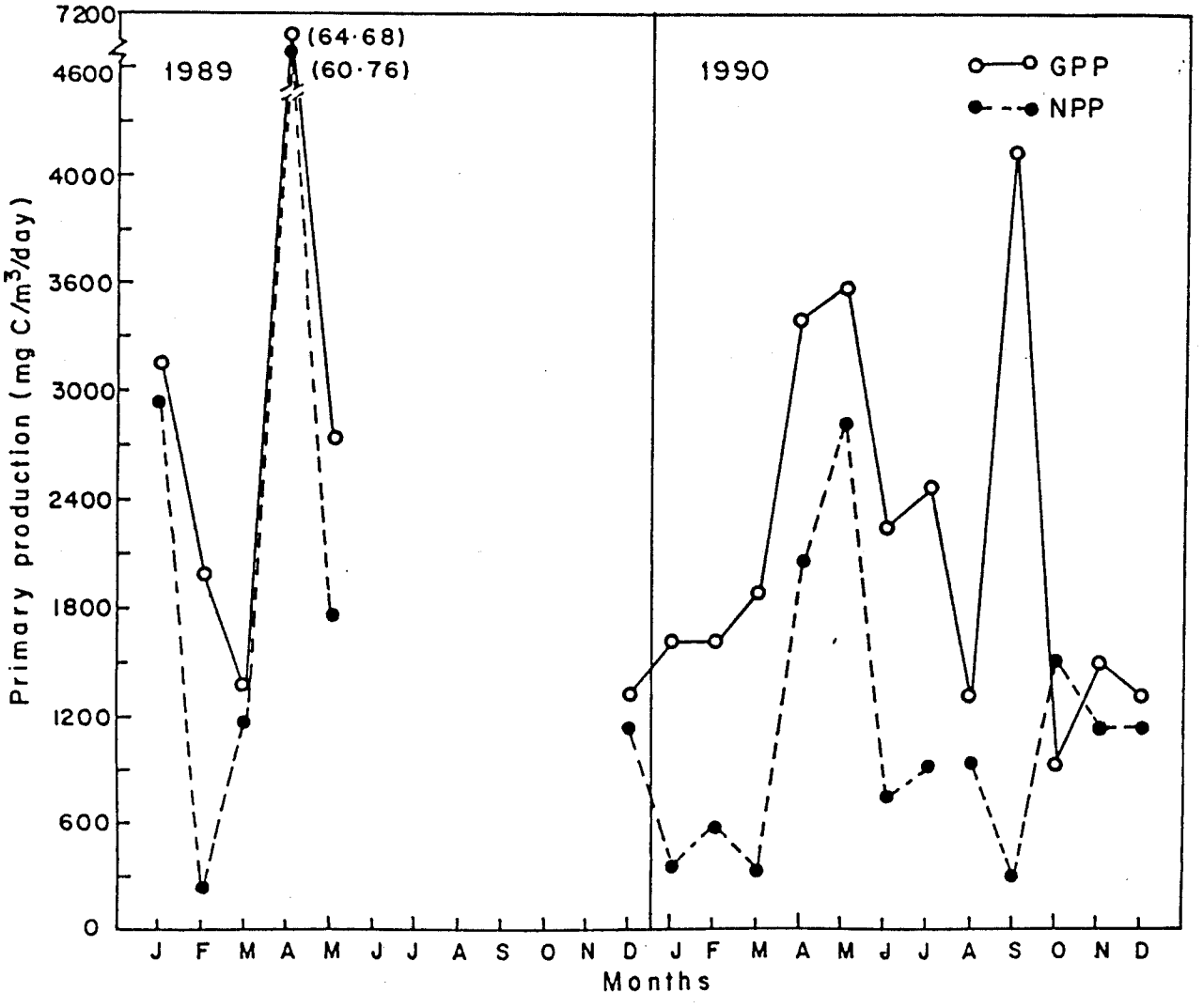

Fig. 22. Monthly variations in gross and net primary production in seasonal pond at Narakkal during 1989 and 1990.

Gradual increase in values were there from January to April 1990. A decrease was observed throughout the monsoon months except in September and September recorded the highest peak (4324 mgC/m^3/day). A sharp decline was noticed in the month of October the value being 940 mgC/m^3/day.

Maximum seasonal mean was obtained in pre-monsoon and the minimum was in post-monsoon season (Table 9).

During 1989 the recorded net primary production values ranged from 196 mgC/m^3/day in February to 6076 mgC/m^3/day in April. The range during 1990 was 376 mgC/m^3/day to 2820 mgC/m^3/day. The lowest values were recorded in January and March and the highest was obtained in May.

The seasonal mean for gross primary production was minimum in post-monsoon and the maximum was in pre-monsoon (Table 9) for net primary production, seasonal mean maximum was obtained in pre-monsoon and the minimum was in monsoon.

Like perennial pond no significant variation was noticed for the primary productivity (gross and net production) over seasons (Table 26 C & D) ($P > 0.05$).

I. CHLOROPHYLL 'a'

(i) Perennial Pond

The density of chlorophyll 'a' varied between 2.196 $mgChl/m^3$ in July and 25.642 $mgChl/m^3$ in June (Fig. 23) from February to April

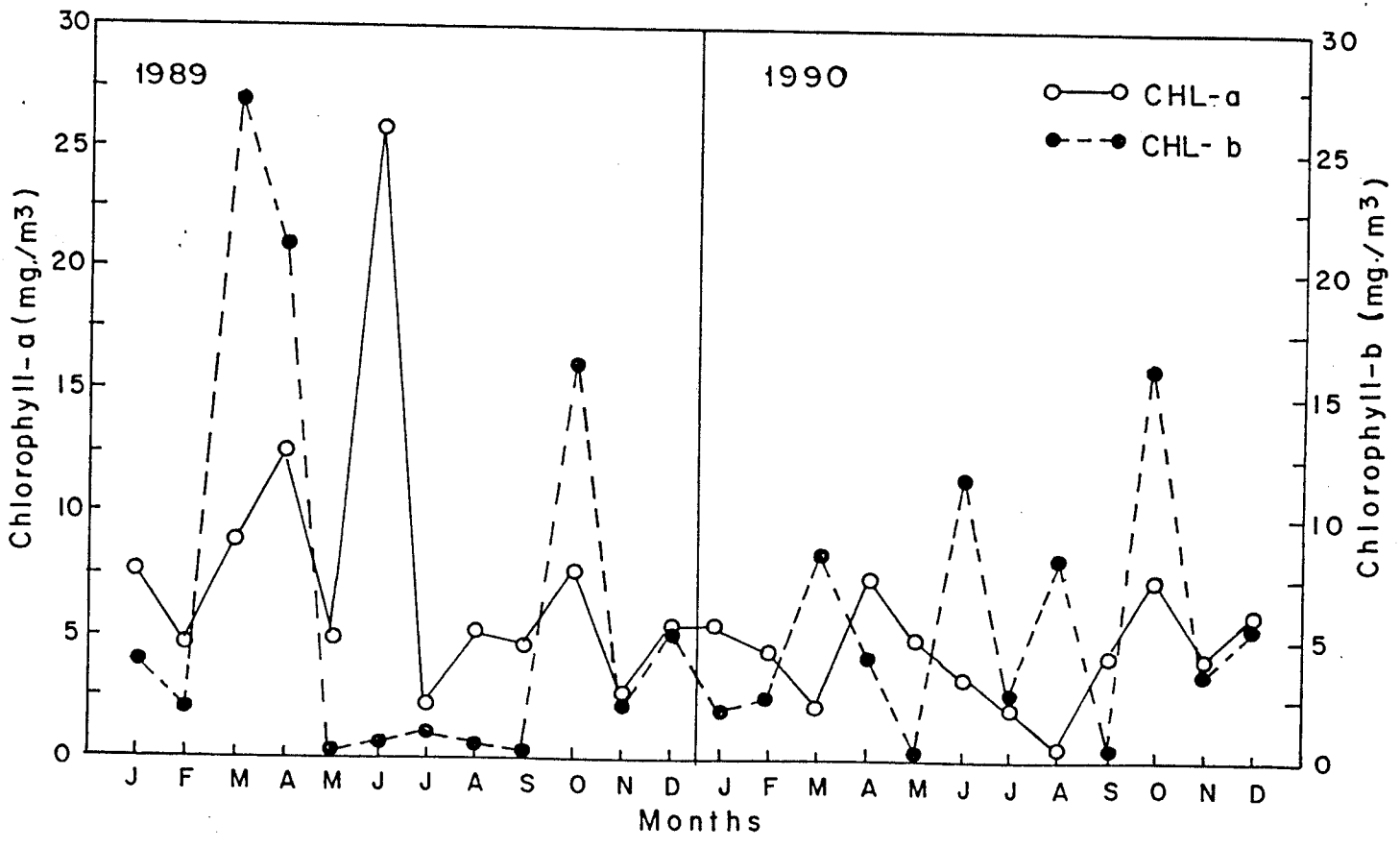

Fig. 23. Monthly variations of chlorophyll-a and chlorophyll-b in perennial pond at Narakkal during 1989 and 1990.

a minor increase occurred in the pigment concentration. A drastic increase was noted in June and a sharp decline was recorded in July and thereafter only slight fluctuation was observed in the distribution of Chlorophyll 'a'.

In 1990 Chlorophyll 'a' concentration ranged from 0.474 mgC/Chl/m^3 in August 7.503 mg Chl/m^3 in October. From April the value decreased gradually for 4 months upto August and minor increase was recorded and the values fluctuated slightly upto December.

Seasonal mean was maximum during pre-monsoon and the minimum was obtained in post-monsoon (Table 10).

The results of ANOVA (Table 27 A) for chlorophyll-a showed no significant variation over seasons ($P > 0.05$).

(ii) Seasonal Pond

During 1989, the highest peak was observed 73.2 mg Chl/m^3 in March. Lowest value was obtained in January 12.51 mg Chl/m^3 (Fig. 24). The lowest range of chlorophyll 'a' 3.476 mg Chl/m^3 was recorded in June and the highest was 35.066 mg Chl/m^3. After a sharp decline in June the concentration increased gradually till the end of the year.

The minimum seasonal mean was obtained in monsoon and maximum was recorded in pre-monsoon (Table 11).

Like perennial pond no significant variation was noticed for chlorophyll-a over seasons (Table 28 A) ($P > 0.05$).

Table 10. Seasonal mean and standard deviation for Chlorophyll pigments in perennial pond

Parameters	Seasons		
	Pre-monsoon	Monsoon	Post-monsoon
PERENNIAL POND			
Chlorophyll-a (mg/m^3)	6.22	5.99	5.79
	± 3.21	± 8.09	± 1.81
Chlorophyll-b (mg/m^3)	8.16	3.01	6.77
	±10.19	± 4.36	± 5.79
Chlorophyll-c (mg/m^3)	9.26	5.48	11.61
	±15.64	± 8.14	±15.75
Carotenoids (mg/m^3)	8.76	2.31	4.27
	± 9.65	± 0.96	± 5.20

Table 27. One-way ANOVA for Chlorophyll pigments in perennial pond during different seasons.

	SOURCE	DF	SS	MSS	F	REMARKS
	PERENNIAL POND					
A.	Seasons	2	0.685	0.342	0.01	NS
	Error	21	553.534	26.359		
	Total	23	554.219			
B.	Seasons	2	113.491	56.746	1.09	NS
	Error	21	1094.864	52.136		
	Total	23	1208.355			
C.	Seasons	2	152.993	76.496	0.41	NS
	Error	21	3912.973	186.380		
	Total	23	4066.966			
D.	Seasons	2	174.804	87.402	2.16	NS
	Error	21	849.230	40.440		
	Total	23	1024.034			

A - Chlorophyll-a, B - Chlorophyll-b, C - Chlorophyll-c,

D - Carotenoids

NS - Not Significant

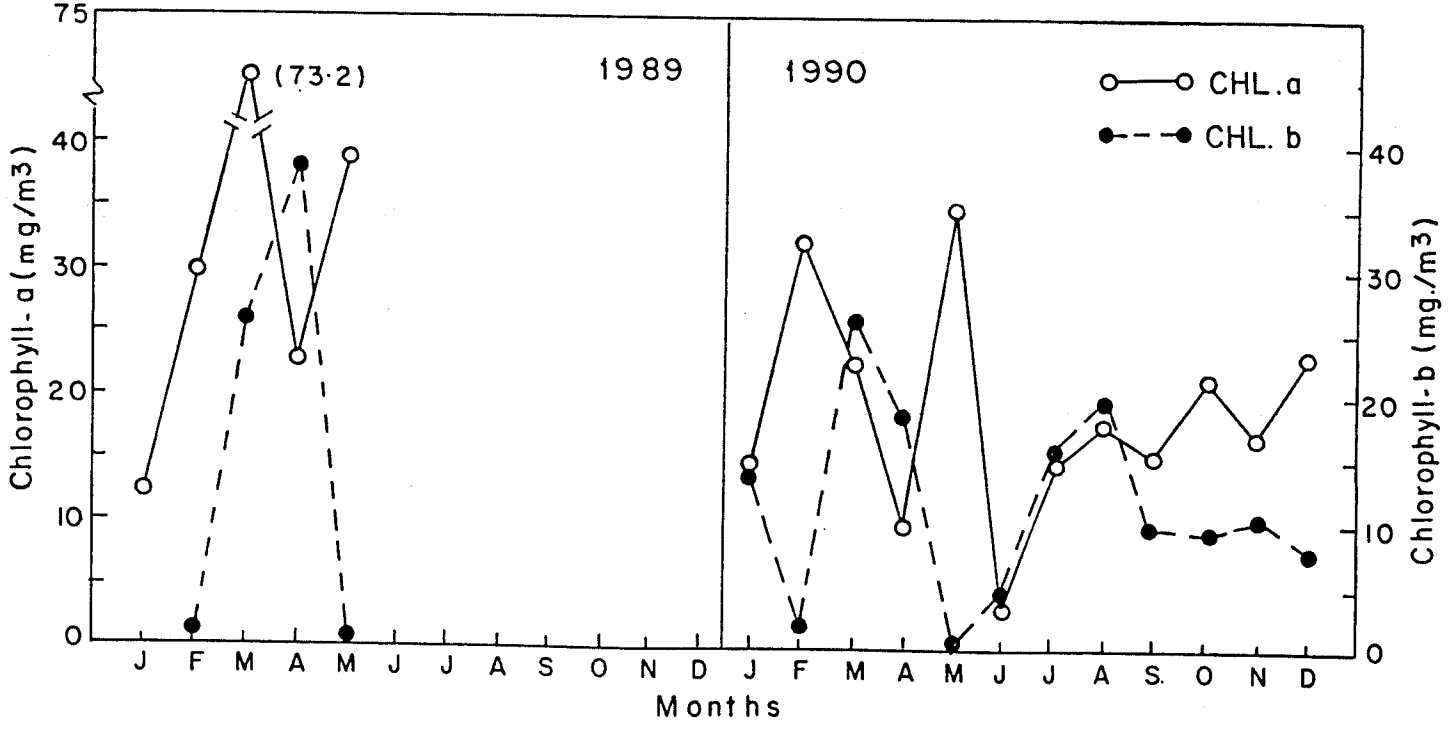

Fig. 24. Monthly variations of chlorophyll-a and chlorophyll-b in seasonal pond at Narakkal during 1989 and 1990.

Table 11. Seasonal mean and standard deviation for Chlorophyll pigments in seasonal pond

Parameters	Seasons		
	Pre-monsoon	Monsoon	Post-monsoon
SEASONAL POND			
Chlorophyll-a (mg/m^3)	33.34 ± 18.46	12.98 ± 6.47	17.89 ± 4.57
Chlorophyll-b (mg/m^3)	14.15 ± 14.82	12.53 ± 6.74	10.30 ± 2.57
Chlorophyll-c (mg/m^3)	22.73 ± 22.10	8.53 ± 5.81	6.21 ± 1.85
Carotenoids (mg/m^3)	21.55 ± 18.53	10.84 ± 6.47	16.48 ± 11.05

Table 28. One-way ANOVA for chlorophyll pigments in seasonal pond during different seasons.

	SOURCE	DF	SS	MSS	F	REMARKS
	SEASONAL POND					
A.	Seasons	2	1370.000	685.032	3.70	NS
	Error	14	2593.000	185.282		
	Total	16	3964.000			
B.	Seasons	2	39.079	19.540	0.15	NS
	Error	13	1694.602	130.334		
	Total	15	1733.681			
C.	Seasons	2	1027.591	513.796	2.04	NS
	Error	14	3534.623	252.473		
	Total	16	4562.214			
D.	Seasons	2	313.731	156.866	0.73	NS
	Error	14	3017.563	215.540		
	Total	16	331.294			

A - Chlorophyll-a, B - Chlorophyll-b, C - Chlorophyll-c,

D - Carotenoids

NS - Not Significant

II. CHLOROPHYLL 'b'

(i) Perennial Pond

Seasonal changes in Chlorophyll 'b' concentration are given in (Fig. 23). During 1989 lowest value of 0.007 was obtained in September and highest primary peak (26.85 mg Chl/m^3) was recorded in March. The quantity of chlorophyll 'b' decreased sharply and the secondary peak occurred in April and May and the values were very similar from May to September. The teritary peak was recorded in October and again the value decreased gradually in November and December.

Three peaks were recorded during 1990. The primary peak was observed in October, secondary peak in June and tertiary peak in March and August and the value ranged from 0.259 mg Chl/m^3 to 15.768 mg Chl/m^3.

Maximum seasonal mean was recorded in pre-monsoon seasonal and minimum was obtained in monsoon (Table 10).

ANOVA (Table 27 B) for chlorophyll-b indicates no significant variation over seasons ($P > 0.05$).

(ii) Seasonal Pond

Chlorophyll 'b' was highest in April 37.815 mg Chl/m^3 and the lowest was recorded during May the value being 0.233 mg Chl/m^3.

In 1990 the lowest range was 0.8575 in May and the highest was recorded (26.061 mg Chl/m^3) in March. The primary peak was observed

in February, the value decreased in April and gradual increase was extended to August and was constant upto December.

Seasonal mean for chlorophyll 'b' was maximum during pre-monsoon 14.15 (± 14.82) and the minimum seasonal mean was in post-monsoon (10.3 ± 2.5) (Table 11).

In seasonal pond, ANOVA (Table 28 B) for chlorophyll-b showed no significant variation over different seasons ($P > 0.05$).

III. CHLOROPHYLL 'c'

(i) Perennial Pond

Seasonal changes in the chlorophyll 'c' are given in Fig.25. The chlorophyll 'c' in perennial pond ranged between 0.1375 mg Chl/m^3 in August to 48.347 mg Chl/m^2 in January during 1989. In January, the highest primary peak occurred and declined sharply during February and subsequently secondary peak occurred in March and declines again in April and May. There was an increase in the value of chlorophyll 'c' during June and October. Only very low values were obtained in rest of the months.

During 1990 the values ranged from 0.2628 mg Chl/m^3 in September to 24.126 mg Chl/m^3 in July. Primary peak was observed during July, secondary peak occurred in January and thereafter the values decreased and the intensity was somewhat constant till June and sharp increase was observed in July. The values increased in October and declined in November and December.

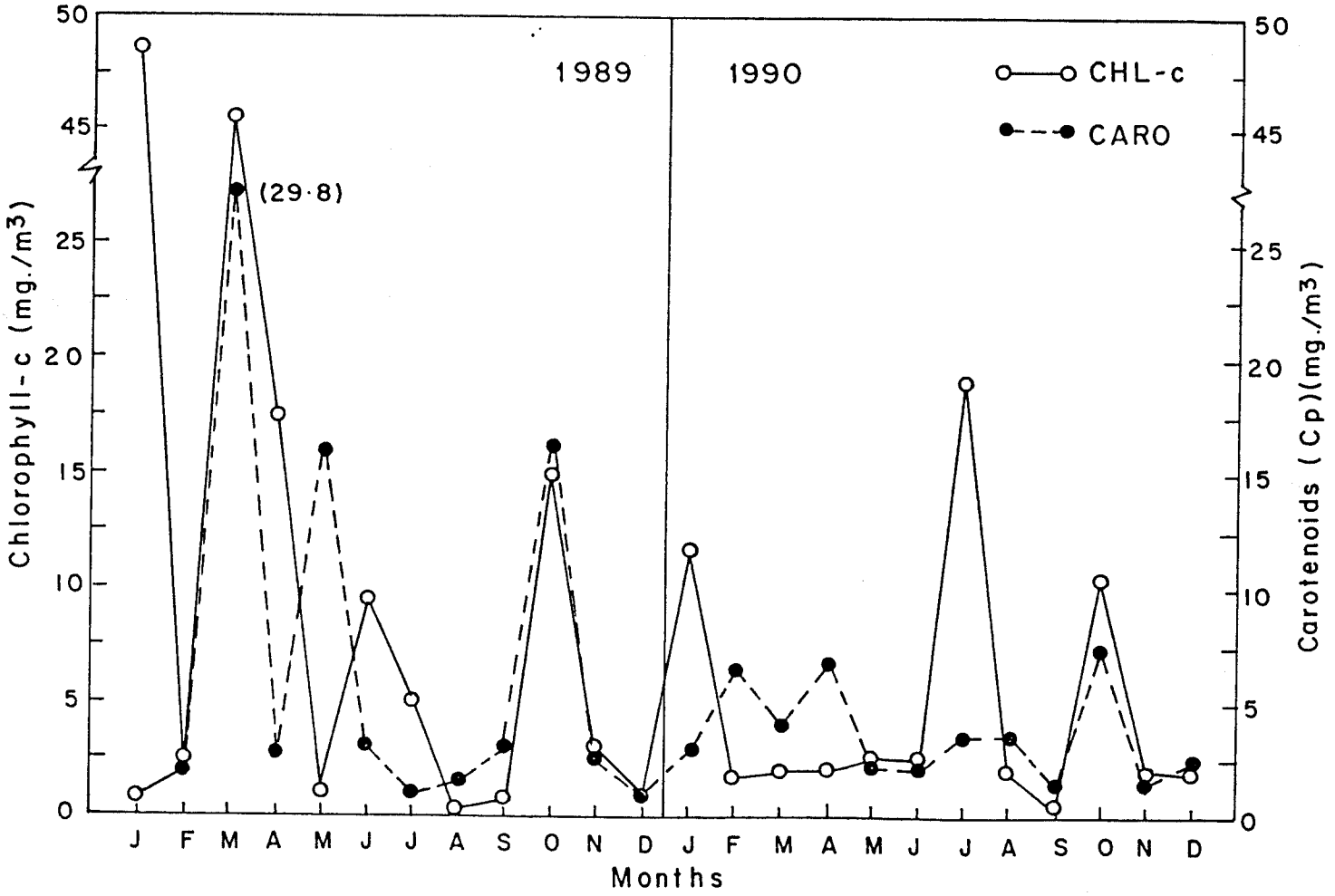

Fig. 25. Monthly variations of chlorophyll-c and carotenoids in perennial pond at Narakkal during 1989 and 1990.

Seasonal mean was found to be maximum in post-monsoon and minimum in monsoon (Table 10).

The results of analysis of variance (Table 27 C) for chlorophyll 'c' implies no significant variation over seasons ($P > 0.05$).

(ii) Seasonal Pond

Chlorophyll 'c' ranged between 4.7485 mg Chl/m^3 to 61.981 mg Chl/m^3 during 1989 (Fig. 26). The primary peak was recorded during March and the secondary peak followed in April and again it declines in May.

During 1990 the values ranged from 0.1653 mg Chl/m^3 in September and 24.118 mg Chl/m^3 in April. A gradual increase was observed from January to April and the values declined in May, July, September and also in November.

The maximum seasonal mean was obtained during pre-monsoon and minimum was in post-monsoon season (Table 11).

Like perennial pond no significant variation was noticed for chlorophyll 'c' over seasons (Table 28 C) ($P > 0.05$).

IV. CAROTENOIDS

(i) Perennial Pond

During 1989, three peaks were observed, the primary was in March the value being 29.88 mg Chl/m^3 and the secondary peak occurred during October (16.153 mg Chl/m^3) and the tertiary one was in May, the value

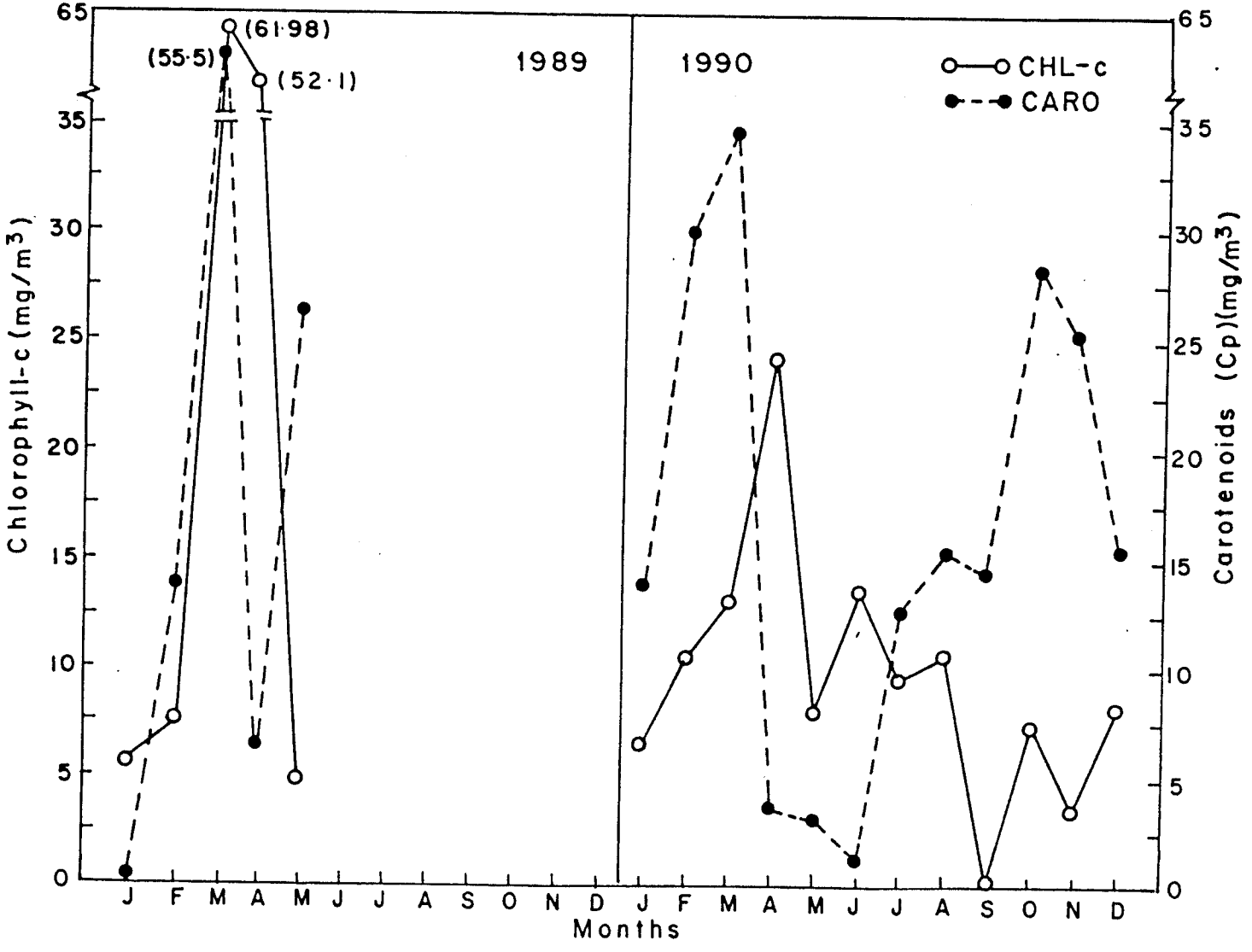

Fig. 26. Monthly variations of chlorophyll-c and carotenoids in seasonal pond at Narakkal during 1989 and 1990.

being 15.972 mg Chl/m^3 (Fig. 25). The carotenoid pigment value ranged from 0.70 - 29.8 mg Chl/m^3. The lowest value occurred during January and July 1989.

During 1990, the highest value (7.13 mg Chl/m^3) was observed during October and the lowest value (1.494 mg Chl/m^3) occurred during September. Compared with previous year much fluctuation was not observed in the seasonal cycle of carotenoid in the perennial pond.

The maximum seasonal mean was obtained in pre-monsoon the value being 8.76 (± 9.65). The minimum 2.31 (± 0.96) was obtained during monsoon (Table 10).

(iii) Seasonal Pond

The carotenoid pigments ranged between 0.071 mg Chl/m^3 in January, the concentration gradually increased upto March (55.56 mg Chl/m^3) being the highest peak during 1989 (Fig. 26). There was a sharp decrease in April (6.169 mg Chl/m^3) and again the concentration showed an increasing trend in May (26.176 mg Chl/m^3).

During 1990, the value of carotenoids ranged between 1.259 mg Chl/m^3 in June and 34.539 mg Chl/m^3 in March. The highest peak was observed in March and declined sharply during April to June and again there was a trend in increase of concentration and extending to the secondary peak in October and decreasing trend was noticed in November and December.

Maximum seasonal mean was obtained in pre-monsoon the value being 21.55 (± 22.10) and the minimum was in monsoon 10.84 (± 6.47) (Table 11).

The results of ANOVA (Tables 27 D, 28 D) for carotenoids showed no significant variation over seasons ($P > 0.05$).

PART - 3 (c) ASSESSMENT OF THE STATUS OF PRODUCTION

NP: GP RATIO

Calculated monthly ratio of net primary productivity to gross primary productivity for the two culture systems during 1989 and 1990 represented in Table 12.

(i) Perennial Pond

The computed ratio was found to range from 0.166 & 0.833 during 1989 and 0.333 to 0.8 during 1990 in the perennial pond.

(ii) Seasonal Pond

The values ranged between 0.1 to 0.939 in 1989 and between 0.2 to 1.6 in 1990.

PART - 3 (d) RATIO OF CHLOROPHYLL PIGMENTS

The ratio of chlorophyll 'b'/'a' during 1989 and 1990 is presented in Table 13, chlorophyll 'c'/'a' in Table 14 and Carotenoids/Chlorophyll 'a' in Table 15. In the present study chlorophyll 'a' concentration always exceeded that of chlorophyll 'b', 'c' and carotenoids. The chlorophyll 'b' 'a' ratio was found high (3.05) in March 1989, 17.56 in August 1990 at

Table 12: Primary Productivity NP/GP (Ratio)

Months	1989						1990					
	Perennial Pond			Seasonal Pond			Perennial Pond			Seasonal Pond		
	GP	NP	NP/GP	GP	NP	NP/GP	GP	NP	NP/GP	GP	NP	NP/GP
	mg C/m^3/day			mg C/m^3/day			mg C/m^3/day			mg C/m^3/day		
January	1764	1176	0.666	3136	2940	0.938	564	376	0.667	1316	376	0.286
February	1176	784	0.667	1960	196	0.100	1692	940	0.556	1316	564	0.429
March	588	392	0.666	1372	1176	0.857	940	376	0.400	1880	376	0.200
April	392	196	0.500	6468	6076	0.939	2820	2256	0.800	3384	2068	0.611
May	1176	392	0.333	2744	1764	0.643	1128	376	0.333	3572	2820	0.789
June	1960	1176	0.600				564	374	0.663	2256	752	0.333
July	980	588	0.600				2444	940	0.385	2444	940	0.385
August	980	196	0.200				2632	2068	0.786	1316	940	0.714
September	1176	196	0.166	- NO DATA -			752	564	0.750	4324	282	0.065
October	588	196	0.333				1316	740	0.562	940	1504	1.600
November	588	392	0.666				1504	564	0.375	1504	1126	0.749
December	1176	980	0.833				1692	1128	0.667	1316	1128	0.857

Table. 13: The ratio of Chlorophyll 'b' / Chlorophyll 'a'

Months	1989						1990					
	Perennial Pond			Seasonal Pond			Perennial Pond			Seasonal Pond		
	Chl 'a' (mg/m^3)	Chl 'b'	Chl 'b'/Chl 'a'	Chl 'a' (mg/m^3)	Chl 'b'	Chl'b'/Chl'a'	Chl'a' (mg/m^3)	Chl'b'	Chl'b'/Chl 'a'	Chl'a' (mg/m^3)	Chl'b'	Chl'b'/Chl'a'
January	7.727	3.898	0.504	12.510	-	0.000	5.415	2.013	0.372	14.840	13.711	0.924
February	4.697	1.892	0.403	30.269	1.451	0.048	4.341	2.632	0.606	32.549	1.874	0.057
March	8.801	26.850	3.050	73.166	25.871	0.354	2.173	8.5	3.912	23.217	26.061	1.230
April	12.441	20.944	1.683	23.284	37.815	1.624	7.362	4.102	0.557	9.949	18.662	1.875
May	4.836	0.068	0.014	39.230	0.332	0.008	5.088	0.259	0.051	35.066	0.857	0.024
June	25.642	0.068	0.002				3.433	11.312	3.295	3.476	4.660	1.306
July	2.196	0.935	0.425				2.077	2.693	1.296	14.929	15.739	1.054
August	5.134	0.358	0.069				0.474	8.325	17.560	18.011	20.029	1.112
September	4.797	0.007	0.002		- NO DATA -		4.192	0.404	0.096	15.509	9.708	0.626
October	7.706	16.153	2.096				7.503	15.768	2.101	21.469	9.031	0.421
November	2.638	2.324	0.881				4.260	3.551	0.834	17.118	10.536	0.615
December	5.293	5.180	0.978				5.843	5.303	0.907	23.539	7.928	0.337

Table. 14: The ratio of Chlorophyll 'c' / Chlorophyll 'a'

Months	1989						1990					
	Perennial Pond			Seasonal Pond			Perennial Pond			Seasonal Pond		
	Chl 'a' (mg/m^3)	Chl 'c' (mg/m^3)	Chl'c'/Chl'a'	Chl 'a' (mg/m^3)	Chl 'c' (mg/m^3)	Chl'c'/Chl 'a'	Chl 'a' (mg/m^3)	Chl 'c' (mg/m^3)	Chl 'c'/Chl'a'	Chl'a' (mg/m^3)	Chl'c' (mg/m^3)	Chl'c'/Chl'a'
January	7.727	48.347	6.256	12.510	5.539	0.443	5.415	11.597	2.142	14.840	6.466	0.436
February	4.697	2.29	0.487	30.269	7.414	0.245	4.341	1.723	0.396	32.549	10.383	0.319
March	8.801	45.568	5.177	73.166	61.981	0.847	2.173	1.913	0.880	23.217	13.151	0.566
April	12.441	17.353	1.394	23.284	52.034	2.235	7.362	1.963	0.267	9.949	24.112	2.423
May	4.836	0.792	0.163	39.230	4.749	0.121	5.088	2.501	0.492	35.066	8.021	0.229
June	25.642	9.269	0.361				3.433	2.501	0.728	3.476	13.539	3.894
July	2.196	5.036	2.293				2.077	24.126	11.611	14.929	9.765	0.654
August	5.134	0.137	0.026				0.474	1.930	4.072	18.011	10.662	0.592
September	4.797	0.6023	0.126	- NO DATA -			4.192	0.263	0.063	15.509	0.165	0.011
October	7.706	14.824	1.923				7.503	10.342	1.378	21.469	7.343	0.342
November	2.638	2.967	1.124				4.260	1.809	0.425	17.118	3.446	0.201
December	5.293	1.03	0.195				5.843	1.982	0.339	23.539	8.277	0.356

perennial pond is indicative of the occurrence of bloom of blue algae. Similar cases of high chlorophyll 'c'/'a' ratio and carotenoids/chlorophyll 'a' observed in perennial pond in January and March 1989 and in July, August 1990 were due to the bloom of dinoflagellates (Table 15).

PART - 4 TOTAL AEROBIC HETEROTROPHS

The annual distribution of total heterotrophic bacteria for the study period is given in Table 16, Fig. 27 and 28. These data were determined by using standard techniques of serial dilution and pourplate method in water and sediment during 1989 and 1990. No data were collected during June to December 1989 due to paddy cultivation in the seasonal pond.

(i) Perennial Pond

WATER

The Fig. 27 reveals that the highest count 256 x 10^5/ml occurred in March 1989 and 205 x 10^5/ml during March 1990. Lowest count was observed 12 x 10^6/ml during October in 1989 and November 1990. High concentrations developed in each year during pre-monsoon months and the decrease in counts was accomplished with large fluctuations during monsoon and post-monsoon months. The ratio between maximum and minimum was 21.3 during 1989 and 17.08 in 1990.

Seasonal mean was maximum during pre-monsoon and minimum was obtained in monsoon (Table 16).

Table. 15: The ratio of Carotenoid/Chlorophyll 'a'

Months	1989						1990					
	Perennial Pond			Seasonal Pond			Perennial Pond			Seasonal Pond		
	Chl 'a' (mg/m^3)	Carotenoid (mg/m^3)	Carotenoid/ Chl 'a'	Chl 'a' (mg/m^3)	Carotenoid (mg/m^3)	Carotenoid/ Chl 'a'	Chl'a' (mg/m^3)	Carotenoid (mg/m^3)	Carotenoid/ Chl 'a'	Chl'a' (mg/m^3)	Carotenoid (mg/m^3)	Carotenoid/ Chl 'a'
January	7.727	0.700	0.091	12.510	0.071	0.006	5.415	2.906	0.536	14.840	13.725	0.925
February	4.677	2.131	0.453	30.269	13.710	0.453	4.341	6.441	1.483	32.549	30.147	0.926
March	8.801	29.880	3.395	73.166	55.560	0.759	2.173	4.080	1.878	23.217	34.539	1.488
April	12.441	2.713	0.218	23.284	6.169	0.265	7.362	6.661	0.905	9.949	3.312	0.333
May	4.836	15.972	3.303	39.230	26.176	0.667	5.088	2.213	0.435	35.066	2.803	0.079
June	25.642	2.839	0.111				3.433	2.213	0.645	3.476	1.259	0.362
July	2.196	0.876	0.398				2.077	3.390	1.632	14.929	12.707	0.851
August	5.134	1.338	0.261				0.474	3.431	7.238	18.011	15.197	0.844
September	4.797	2.936	0.612		- NO DATA -		4.192	1.494	0.356	15.509	14.206	0.916
October	7.706	16.153	2.096				7.503	7.131	0.950	21.469	28.128	1.310
November	2.638	2.324	0.881				4.260	1.509	0.354	17.118	25.162	1.469
December	5.293	1.030	0.195				5.843	2.418	0.414	23.539	15.356	0.652

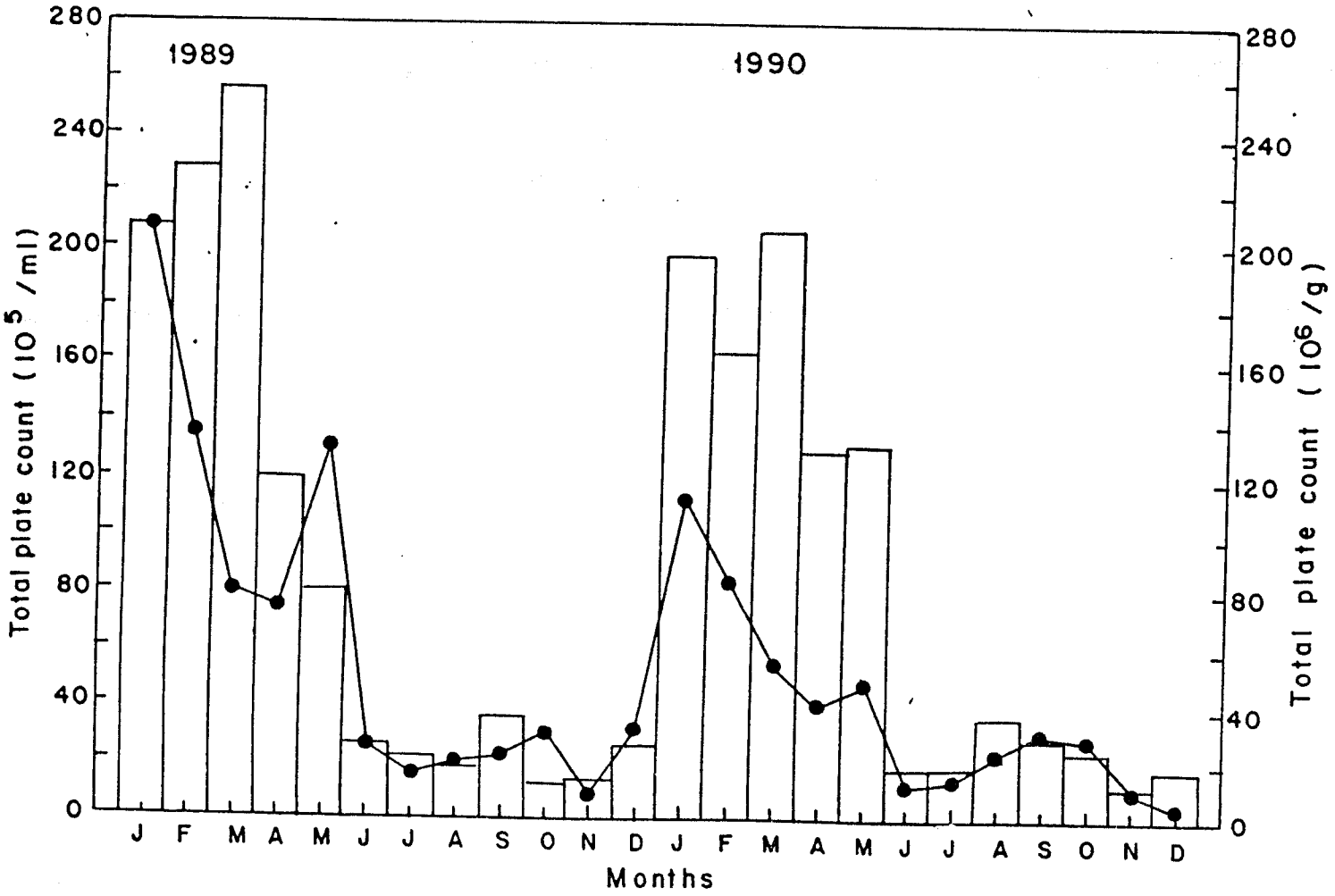

Fig. 27. Monthly variations of total plate count in water and sediment samples of perennial pond at Narakkal during 1989 and 1990.

Table 16. Seasonal mean and standard deviation for the total heterotrophs in water and sediment at perennial and seasonal ponds.

Parameters	Seasons		
	Pre-monsoon	Monsoon	Post-monsoon
PERENNIAL POND			
Total heterotrophs in water (10^5/ml)	164.25 ± 60.48	24.5 ± 7.71	63.67 ± 85.91
Total heterotrophs in Sediment (10^6/g)	80.0 ± 36.04	19.5 ± 6.18	53.5 ± 71.42
SEASONAL POND			
Total heterotrophs in water (10^5/ml)	93.37 ± 53.99	18.5 ± 5.45	58.66 ± 47.41
Total heterotrophs in sediment (10^6/g)	62.5 ± 37.5	18.75 ± 4.35	88.33 ± 78.5

ANOVA for the total heterotrophs count in water, between pre-monsoon and monsoon (SE=30.413) pre-monsoon and post-monsoon (SE=413) differed significantly (Table 29 A) ($P < 0.01$).

SEDIMENT

The concentration of viable total heterotrophs in sediment (Fig.27) ranged from 7 x 10^6/gm in November 1989 to 208 x 10^6/gm in January. During 1990 the viable count ranged 3 x 10^6/gm in December and highest was recorded as 112 x 10^6/gm in January.

The ratio between maximum and minimum was 37.3 which indicated large fluctuation in the distribution of viable total counts.

During 1990 the bacterial concentration decreased gradually in pre-monsoon months and at the onset of pre-monsoon (May 1990) it increased into 47 x 10^6/gm. The intensity declined till July because of monsoonal effect. There was an increasing trend in concentration observed in August, September and October and again a drastic decline occured in November and December, whereas in 1989 the increasing values were accomplished with large fluctuations having peaks in February (135 x 10^6/gm) and May (129 x 10^6/gm) and from there slight variation occurred till December 1989. The ratio between maximum and minimum was 29.7.

The seasonal mean was maximum during pre-monsoon (80.0 ± 36.04) and minimum was in monsoon (19.5 ± 6.18), (Table 16).

Table 29. One-way ANOVA for total plate count of water and sediment in perennial and seasonal ponds during different seasons.

	SOURCE	DF	SS	MSS	F	REMARKS
	PERENNIAL POND					
A.	Seasons	2	83163.250	41581.625	11.24	HI.SIG (1%)
	Error	21	77693.375	3699.685		
	Total	23	160856.625			
B.	Seasons	2	14716.000	7358.000	3.43	NS
	Error	21	45064.000	2145.000		
	Total	23	59780.000			
	SEASONAL POND					
C.	Seasons	2	15330.289	7665.145	3.62	NS
	Error	15	31734.211	2115.614		
	Total	17	47064.500			
D.	Seasons	2	11638.195	5819.098	2.14	NS
	Error	15	40714.086	2714.272		
	Total	17	52352.281			

A & C - TPC in water, B & D - TPC in sediments

HI.SIG - Highly Significant

NS - Not Significant

ANOVA for the total heterotrophs count in sediment, between pre-monsoon and monsoon differed significantly (SE=23.162), (Table 29 B) ($P > 0.05$).

(ii) Seasonal Pond

WATER

In seasonal pond the highest peak in concentration occured during 1989 March (176 x 10^5/ml) and the lowest was recorded 31 x 10^5/ml in December (Fig. 28).

The increases in concentration was evident during the onset of pre-monsoon. The ratio between maximum and minimum was 5.67.

During 1990 highest concentration apparently occurred in February the value being 152 x 10^5/ml in water and the lowest concentration was recorded in August. The fluctuation varied from season to season. The ratio between maximum and minimum was 12.6.

The seasonal mean was maximum during pre-monsoon (93.37 ± 53.99), minimum was in monsoon (18.5 ± 5.45), (Table 16).

The total heterotrophic count, in water between pre-monsoon and monsoon differed significantly (SE=28.167), (Table 29 C) ($P > 0.05$).

SEDIMENT

The total heterotrophic bacterial concentration in sediment was found to increase in January and March (Fig. 28) during 1989. The ratio between maximum and minimum was 6.

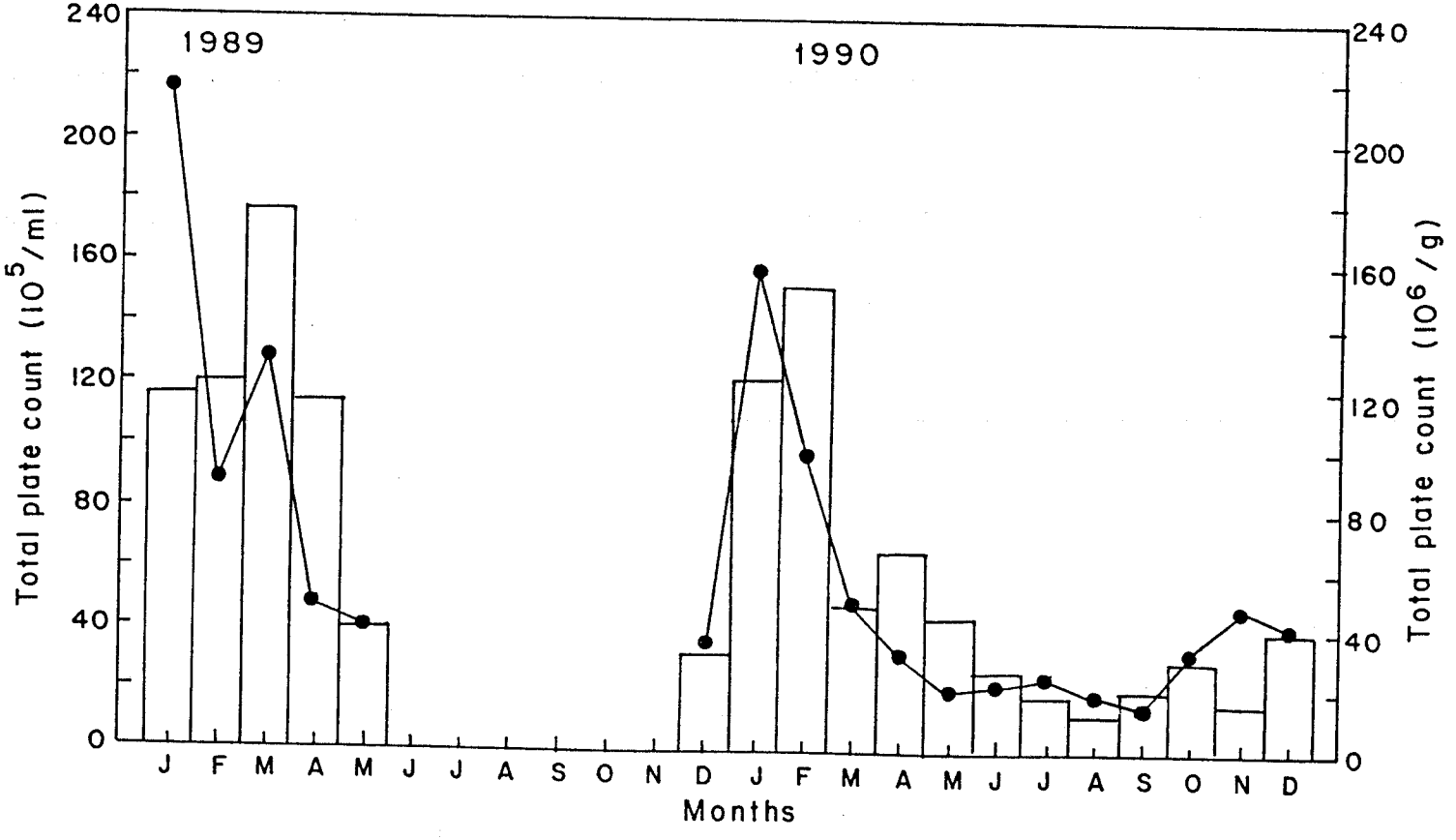

Fig. 28. Monthly variations of total plate count in water and sediment samples of seasonal pond at Narakkal during 1989 and 1990.

During 1990, the recorded values accomplished with large fluctuations having higher peaks in January and February and decreasing trend followed in monsoon and post-monsoon months. The ratio between maximum and minimum was 12.

The maximum seasonal mean was obtained in post-monsoon (88.33 ± 78.5), and minimum during monsoon (18.75 ± 4.35), (Table 16).

ANOVA (Table 29 D) for the total heterotrophs counts in sediment over seasons, between water and sediment (Table 32 C & F) was found no significant variation ($P > 0.05$).

Table 32. One-way ANOVA for the temperature, pH and total aerobic heterotrophs in the perennial and seasonal ponds between water and sediment.

	SOURCE	DF	SS	MS	F	REMARKS
	PERENNIAL POND					
A.	Space	1	1.332	1.332	0.34	NS
	Error	46	180.480	3.923		
	Total	47	181.813			
B.	Space	1	0.384	0.383	2.00	NS
	Error	46	8.831	0.192		
	Total	47	9.214			
C.	Space	1	13035.031	13035.031	2.73	NS
	Error	46	219492.953	4771.586		
	Total	47	232527.984			
	SEASONAL POND					
D.	Space	1	0.563	0.563	0.14	NS
	Error	34	133.125	3.915		
	Total	35	133.688			
E.	Space	1	0.255	0.255	0.46	NS
	Error	34	18.830	0.554		
	Total	35	19.085			
F.	Space	1	128.438	128.438	0.04	NS
	Error	34	99416.781	2924.023		
	Total	35	99545.219			

A & D - Temperature, B & E - pH

C & F - Total aerobic heterotrophs.

NS - Not Significant

PART - 5 STATISTICAL ANALYSIS

In order to assess the correlation and extent of influence between the parameters studied, the following characters were selected and correlation coefficient 'r' was calculated.

1. Temperature
2. Salinity
3. Dissolved oxygen
4. pH
5. Chlorophyll-a
6. Primary productivity
7. Total aerobic heterotrophs
8. T_t of glucose
9. T_t of acetate
10. V_{max} of glucose
11. V_{max} of acetate
12. $K_t + S_n$ of glucose
13. $K_t + S_n$ of acetate

The result of these parameters during 1989 & 1990 in the perennial pond are presented as correlation matrix in Table 33.

Temperature was positively correlated with V_{max} of acetate (r=0.5986) salinity was positively correlated with total aerobic heterotrophs (r=0.7755). Dissolved oxygen was negatively correlated with chlorophyll-a (r=-.3976) and total aerobic heterotrophs (r=-.5064) pH was positively correlated with K_t+S_n of acetate (r=0.4750) Chlorophyll-a was positively correlated

Table 33. Correlation Matrix of parameters in perennial pond.

	1	2	3	4	5	6	7	8	9	10	11	12	13
1.	1.000												
2.	0.3661	1.000											
3.	-.0266	-.3429	1.000										
4.	0.1944	-.3584	0.0339	1.000									
5.	-.1711	0.0661	-.3976*	0.1446	1.000								
6.	0.1633	-.0816	0.1510	-.1003	0.0414	1.000							
7.	0.2384	0.7755*	-.5064*	-.3008	0.0319	0.1033	1.000						
8.	-.0322	0.1738	-.0088	-.0182	-.0124	-.0057	-.0294	1.000					
9.	0.0615	-.3727	0.1634	0.3577	0.4228*	0.1713	-.1555	-.0811	1.000				
10.	0.0857	0.1291	-.2163	0.0450	-.3359	-.1613	0.3600	-.4060*	0.2982	1.000			
11.	0.5986*	0.1411	0.0592	0.0664	-.1570	0.0535	0.0354	-.0131	0.2695	0.2778	1.000		
12.	0.2567	0.1496	-.2182	0.1777	0.2026	-.1312	0.2593	0.2566	0.2241	0.6802*	0.3212	1.000	
13.	0.3172	-.2965	0.2125	0.4750*	-.3787	0.2118	-.1712	-.0468	0.7978*	0.376	0.5023*	0.3152	1.000

* Significant at 5% level

with T_t of acetate (r=0.4228). T_t of glucose was negatively correlated with V_{max} of glucose (r=-.4062). T_t of acetate was positively correlated with K_t+S_n of acetate (r=0.7978). V_{max} of glucose was positively correlated with K_t+S_n of glucose (r=0.6802). V_{max} of acetate was positively correlated with K_t+S_n of acetate (r=0.5023).

Perennial pond, 1989 & 1990

Mean and STD Deviation of different characteristics

CHR		MEAN	SD
1.	Temperature	31.27	2.064
2.	Salinity	12.56	7.837
3.	Dissolved oxygen	5.16	0.911
4.	pH	7.48	0.452
5.	Chlorophyll-a	6.00	4.908
6.	Primary productivity	1274.66	676.709
7.	Total aerobic heterotrophs	83.95	83.33
8.	T_t of glucose	10.10	4.324
9.	T_t of acetate	8.21	3.727
10.	V_{max} of glucose	0.38	0.317
11.	V_{max} of acetate	0.26	0.081
12.	K_t+S_n of glucose	3.06	1.938
13.	K_t+S_n of acetate	2.24	1.434

The results of these parameters during 1989 & 1990 in the seasonal field are presented as correlation matrix in Table 34. Temperature was positively correlated with pH (r=0.7155). Salinity was positively correlated

Table.34. Correlation matrix of parameters in Seasonal pond.

	1	2	3	4	5	6	7	8	9	10	11	12	13
1.	1.000												
2.	0.0884	1.000											
3.	0.3376	0.0146	1.000										
4.	0.7155*	0.1797	0.4242	1.000									
5.	0.0936	0.2999	0.3728	0.3557	1.000								
6.	0.4406	0.1542	0.4470	0.1833	-.2434	1.000							
7.	0.0965	0.6977*	0.0481	0.0884	0.5443*	0.0355	1.000						
8.	0.3554	-.1760	0.3976	0.1582	-.3922	0.3783	-.2569	1.000					
9.	0.0792	0.5264	0.0578	-.2467	-.1712	0.3259	-.3115	0.2619	1.000				
10.	0.1762	0.2371	-.1753	-.1606	0.4012	0.0035	0.4070	-.6676*	0.2194	1.000			
11.	0.4118	-.3252	-.3775	-.4192	-.1964	-.2062	-.3379	-.5961*	0.1164	0.7389*	1.000		
12.	0.4381	0.0307	0.2567	0.2868	-.1766	0.6105*	0.0195	0.5729*	0.4153	-.4509	0.2582	1.000	
13.	0.3113	-.5830*	-.2062	-.5273*	-.2147	-.0326	-.4121	-.1106	0.7603*	0.2562	0.6282*	0.0220	1.000

* Significant at 5% level

with total aerobic heterotrophs (r=0.6977) and negatively correlated with K_t+S_n of acetate (r=-.5830). pH was negatively correlated with K_t+S_n of acetate (r=-.5273). Chlorophyll-a was positively correlated with total aerobic heterotrophs (r=0.5443). Primary production was positively correlated with K_t+S_n of glucose (r=0.6105). T_t of glucose was negatively correlated with V_{max} of glucose (r=-.6676) and V_{max} of acetate (r=0.5961) and positively correlated with K_t+S_n of glucose (r=0.5729). T_t of acetate was positively correlated with K_t+S_n of acetate (r=0.7603). V_{max} of glucose was positively correlated with V_{max} of acetate (r=0.7389). V_{max} of acetate was positively correlated with K_t+S_n of acetate (r=0.6282).

Seasonal pond, 1989 & 1990

Mean and STD Deviation of different characteristics

CHR		MEAN	SD
1.	Temperature	32.25	2.157
2.	Salinity	14.26	7.892
3.	Dissolved oxygen	5.92	1.818
4.	pH	7.75	0.746
5.	Chlorophyll-a	24.01	15.741
6.	Primary productivity	2364.66	1405.51
7.	Total aerobic heterotrophs	65.16	52.616
8.	T_t of glucose	9.925	3.793
9.	T_t of acetate	12.73	3.584
10.	V_{max} of glucose	0.20	0.114
11.	V_{max} of acetate	0.23	0.058
12.	K_t+S_n of glucose	1.58	0.832
13.	K_t+S_n of acetate	2.61	1.093

IV. DISCUSSION

The principal interest of this investigation using tracers was to determine the seasonal and vertical distribution of microbial chemo-organotrophy as measured by glucose and acetate utilisation in aquaculture ponds, Narakkal, Cochin (Kerala, India). The tracer technique was useful in measuring glucose and acetate incorporation rates as low as 0.5 n ML^{-1}. The incorporation followed saturation kinetics. As it has been established that abiotic factors particularly chemical characteristics of the environment exert profound influence on the growth and survival of micro-organisms, attention has been focussed to determine the relationship of certain possible controlling factors to microbial chemo-organotrophy. The data obtained during the two year study period will be discussed under four headings.

Heterotrophy relationships, hydrological related factors, primary productivity - related factors and relationship between total aerobic heterotrophic bacterial distribution.

Following the work of Wright and Hobbie (1966) numerous measurements of uptake kinetics of various substrates by autochthonous natural communities of aquatic micro-organisms have been reported. In most situations the uptake was found to obey a Michaelis Monod relationship.

$$V = \sum_{1} v_i \simeq V_{max}\, S/(S + K_t) \quad \ldots\ldots\ldots (1)$$

Where V is the total rate of uptake,

v_i = is the rate of uptake by population,

i = is the microbial community,

V_{max} = is the maximum total rate of uptake

S = is the substrate concentration, and

K_t = is the half saturation constant of uptake.

The validity of this approach when dealing with heterogenous microbial communities has been discussed by Williams (1973). He showed that the validity of relation (1) is better when the community becomes less diverse. In some cases, departure from Michaelis - Menton - Monod - kinetics have been reported for the uptake of some substrate by natural microorganisms (Vaccaro and Jannasch, 1966; Hamilton and Preslan, 1979; Barvenik and Malloy, 1979); they concern generally oligotrophic environments where it is known (Martin and Bianchi, 1980) that microbial diversity is more important. In all the other cases the validity of relation (1) indicates either that one single microbial strain dominates all the other in the utilisation of the substrates or that in all the strains utilising the substrate has a very similar value of K_t. Therefore, the K_t value obtained from this kind of measurement with natural bacterial communities characterises the affinity towards the substrate of the dominant microbial populations.

For substrates used purely for energy, like glucose and acetate, there appears to be a relationship between the K_t value of the microbial community and its natural rate of substrate utilisation, the lower the flux of the substrate the lower the K_t value. Since the rate of utilisation of the substrate (rather than its concentration) is the best index of the fertility of the aquaculture ponds in terms of this substrate, this trend can be interpreted as reflecting the competition between microorganisms

for their substrates, the lower the availability of a substrate, the higher the affinity for this substrate developed by the enzyme systems.

Williams (1970) found that 49% of radio-active glucose was taken up by organisms passing through a 1.2 μm pore size filter, 68% of the substrate taken up by organisms passing through 3 μm filter, and 80% of the substrate taken up by organisms passing through a 8 μm filter in separate samples. He concluded that bacteria were mainly responsible for most of the uptake of free glucose. According to Azam and Hodson (1977); 90% of the heterotrophic activity results from bacteria passing through 0.1 μm membrane filter. Because of the low number of bacteria that could be cultured from the waters it has been difficult to accept the traditional view that bacteria were the most important users of detrital and dissolved organic material in the sea (Williams, 1975). However, two factors must be taken into consideration to resolve this dilemma-'time' (as evidenced by residence time of water) and the fact that many bacteria in the marine environment do respire but do not form 'colonies' on media (Hoppe, 1976, 1978).

Low concentration of organic matter, age of the organic matter, slow rate of O_2 utilisation coupled with rate of organic matter utilisation, turnover time, residence time of the organic matter are main reasons for studying substrate capcutre by microorganisms in low nutrient environments. The importance of substrate capture in low nutrient environment lies in the reservoir of organic matter in aquaculture ponds.

The pattern for heterotrophy relationships changes of the kinetic parameters T_t, V_{max} and $K_t + S_n$ in the present observation in perennial and seasonal ponds are as follows:

(a) Heterotrophy Relationships

(i) Glucose Utilization

(a) Seasonal variation in the turnover time of glucose (T_t)

In perennial pond the most striking result of the glucose uptake determination was that the turnover time (Fig.3) was maintained by the natural heterotrophic microbes at a uniform minimum during pre-monsoon months (in February 1989 and May 1990) for surface water. The minimum turnover time in surface water indicated the active heterotrophic activity during pre-monsoon months when temperature was recorded highest. While the turnover time remained uniformly low the maximum velocity of uptake and ($K_t + S_n$) varied markedly during the pre-monsoon season. V_{max} and ($K_t + S_n$) of glucose were directly proportional and also found to be significant (Table 33). Since both must increase or decrease in order for the turnover time to remain constant. So the system therefore, seem to operate in such a way to prevent the accumulation of glucose. Allen (1969) reported relatively constant turnover time in lake Lotsjon, Sundbyberg, Sweden for both the substrates. Andrews and Williams (1971) determined turnover rate of glucose (turnover rate is the reciprocal value of the turnover time given in %) in the English channel, where seasonal differences of more than two orders of magnitude were found. Variations of about the same magnitude were observed by Wright and Hobbie (1966) at Lake

Erken (Sweden). It is assumed that the seasonal changes of T_t are mainly caused by changes of actual uptake velocity, and only to a smaller degree by variations of the natural concentrations of the substrate. Thus, the relatively small variation in turnover times at the eutrophic inner Kiel Fjord found out by Gocke (1977) indicates that in eutrophic and in polluted areas the decrease of heterotrophic activity during winter is less pronounced than in less eutrophic areas. Chandramohan and Ramaiah (1987) observed considerable variation in the T_t of glucose both in space and time and generally oceanic water around Kavaratti Atoll exhibited high T_t for glucose. The T_t increased tremendously (30 times) in January compared to in April.

The factor or factors which cause the increase in T_t of glucose during April, September and December 1989 at surface water in perennial pond showed the onset of stratification originating at the surface. The same trend was followed during 1990 also (Fig.3). This was indicated by the fact that surface T_t value showed greater increase or preceeded the decrease at bottom water. In seasonal pond T_t of glucose was negatively correlated with V_{max} of glucose. Gocke (1977) observed horizontal variation in the turnover time for glucose in Kiel Fjord and the central Kiel bright than the vertical variation. He also found somewhat longer turnover time close to the bottom analogous to the maximum uptake velocity. In the present observation also the turnover time and maximum uptake velocities for glucose was found to be analogous. It should be

noted that the actual turnover times are somewhat shorter if the gross uptake is determined. However, this had no effect on the spatial differences. Kirstein (1991) found that the turnover time of glucose reached the smallest value (6 hrs) at late spring till early summer while the largest turnover times (300 hrs) occurred during December-February in Central Kiel bight. This may be the reason for highest number of saprophytes at low turnover times but not at maximum bacterial production. In the present observation the highest turnover time was recorded 18.325 hrs during April 1989 at bottom water and the lowest T_t (3.445 hrs) was recorded in surface water of perennial pond for glucose. Banoub and Williams (1972) found the rate of heterotrophic bacterial turnover of glucose was 30-50% per day. The rate of turnover decreased with depth in the western mediterranean sea. Turnover time in Lake Kinneret ranged from 16 to 625 hrs, and the period April to June was characterised by short turnover time probably due to bacteria involved in the degradation of dead peridinium cells. Ferroni et al. (1983) found the turnover time for glucose increasing with decreasing pH. V_{max} values decreased with decreasing pH. Castilli and Lawrence (1979) examined glucose uptake during post larval period in shrimp and found that bacteria on the carapace accounted for most of the uptake. Sang-Jin-Kim (1991) studied the extracellular enzyme activity in the Bransfield, ANTARCTICA. The turnover times of glucose were in the ranges of 41 to 2094 (hrs). Turnover time of organic matter was extremely variable depending on the sampling station and water depth.

(b) Seasonal variation of maximum uptake velocity of glucose (V_{max}):

The activity of heterotrophic bacteria may be indicated by the magnitude of V_{max} (Hobbie, 1967; Wright and Hobbie, 1966). The glucose uptake (V_{max}) was greatest in pre-monsoon season in surface water of perennial pond and had negative correlation with T_t of glucose. Chandramohan and Ramaiah (1987) also found the velocity was same for all the substrates in the Lagoon waters. V_{max} in perennial pond was four times higher which indicated the V_{max} is affected by other factors like input of fertilizers which will affect inturn the bacterial population. Allen (1969) reported greater activity at 7 meters depth and least at 0.5 m and intermediate at the middle depth. Wetsel (1967) did not observe such trend, but in the present observation V_{max} of glucose was found to be more in surface water than in bottom water. The gross uptake of glucose and T_t was found to be negatively correlated in the surface water of perennial pond of Narakkal. The gross uptake values of glucose in Kiel Fjord (1 m depth) was 0.266 µg C/l/hr (Gocke, 1977) and in the present observation it was 0.45 µg C/l/hr in pre-monsoon months.

The annual average values of the maximum uptake velocity for glucose in Kiel Fjord was 0.028 $\mu gC\ l^{-1}h^{-1}$ (2 m), 0.025 $\mu gC\ l^{-1}h^{-1}$ (10 cm) and 0.034 $\mu gC\ l^{-1}h^{-1}$ (18 mm), whereas Chandramohan and Ramaiah (1987) found 1.165 $\mu gC\ l^{-1}h^{-1}$ which indicated more heterotrophic activity in the coral atolls of Lakshadweep when compared to temperate waters. In the present observation the maximum uptake velocity of glucose are 0.45 $\mu gC\ l^{-1}h^{-1}$, 0.38 $\mu gC\ l^{-1}h^{-1}$ for pre-monsoon, monsoon and post-monsoon

seasons respectively in surface water and bottom water of V_{max} also was found to be significant in surface water of perennial pond. The V_{max} was unchanged during June to November 1990 in bottom water but (K_t + S_n) increased gradually from June to September and again in December 1990. Since V_{max} was unchanged K_t + S_n increased during this period an increase in the substrate concentration was likely. A change in bacterial density or composition of the aerobic hetetrotrophs could tend to change the V_{max}. Otherwise a decrease in velocity is associated with an increase in K_t.

The actual uptake rate of glucose in the surface and bottom water showed seasonal fluctuation. Compared to bottom water, surface water recorded higher uptake rate. Very constant low uptake rates of glucose in the bottom water from April to July and September to December was recorded during 1989 and also during January to July except slight increase in April during 1990. The differences in the V_{max} values in both the year seem to reflect their observed seasonal abundance in the aquaculture ponds.

(c) Seasonal variation of K_t + S_n of glucose

The observed increase in glucose K_t + S_n values in pre-monsoon (Table 2) may be caused by increases in the transport constant (K_t), the natural substrate concentration (S_n) or by simultaneous variations of both the parameters. Since high concentration of dissolved organic compound tend to increase V_{max} levels the observed parallel increase in maximum

uptake velocities and the $K_t + S_n$ values for glucose could be due to higher concentration of this compound (Fig. 7) during April 1989 in perennial pond surface water. It is also possible that when increases of V_{max} were observed distinct bacterial groups with high transport constants could have dominated uptake of glucose. This suggests that it was the uptake constant which was varying and carbon substrate was not the limiting nutrient factor for the bacterioplankton activity. Therefore, the results show perennial pond surface water was enriched nutritionally during the period of observation. The $(K_t + S_n)$ of glucose in perennial pond between surface and bottom water was found to be significant (Table 30 C). In surface water (Table 33) no correlation was found between uptake rate and other environmental parameters. As uptake activity was found independent this is obviously due to broader spectrum of environmental parameters interacting in surface water thus resulting in complex relationship between the physico-chemical parameters and the uptake activity. Erkenurecher and Steevenson (1975) also obtained no relationship between variables and uptake activity. Interactions between variables may depend upon the type of biotope studied and the relationships between biological and chemical variables in different depths of the water column (Bolter et al., 1977).

The exchange of nutrients between the bottom water and surface water above is not a simultaneous one way factor which occur spontaneously. The concentration of glucose in bottom water was relatively high when compared to surface water (Meyer Reil et al., 1978) the bottom

water may be described as a buffer system which responds only slowly to the seasonal fluctuation of bacterial number, biomass and uptake activity in the surface water. During the period (Fig. 3) from March to August and September to November there was a gradual decrease in the turnover time. This was accompanied by a gradual increase (Fig. 7) in $K_t + S_n$ and V_{max} in surface water, of perennial pond. In seasonal pond surface water there was a gradual decrease in the turnover time. This was accompanied by a gradual increase (Fig. 7) in $K_t + S_n$ and V_{max} in surface water, perennial pond. In seasonal pond surface water there was gradual decrease in T_t was recorded from June to August and again September to November during 1990 (Fig. 4). These may be possibly due to the increase, in temperature which would increase V_{max} and while decreasing $K_t + S_n$. A qualitative and quantitative change in the microbial community was also evident. All these changes are probably also due to changing values of K_t and V_{max} to those of a different community. Increases in the T_t at surface and bottom water occurred at the same time during March 1989 which may be due to changing population which developed in surface water and decended through bottom water. The perennial pond values of T_t in rest of the months and also in seasonal pond were significantly different.

The values of V_{max} and $K_t + S_n$ declined gradually during January and from April to July and, also from September to December in 1989. During 1990 the decrease in Kinetic parameters was from January to August except in April (Fig. 5, 6) and also from October to December

in bottom water in perennial pond, whereas in seasonal pond during 1989 the values declined in April and in 1990, it was found to increase in March and also from June to November. This was probably for the same reasons as discussed above. The maximum occurrence of $K_t + S_n$ in August 1989 in bottom water and April 1989 in surface water at perennial pond and during May 1989 in seasonal pond are probably due to high concentration of the substrates. It may also be due to the initiation of aerobic and microaerophilic conditions with a concomitant change in the nature of the population, K_t, and glucose metabolism (Francisco, 1970).

(ii) Acetate Utilization

a) Seasonal variation of the turnover time of acetate (T_t)

The seasonal variations of turnover time V_{max} and $K_t + S_n$ for both the ponds are shown in Fig. 9, 10, 11, 12, 13 & 14 respectively. In perennial pond, bottom water T_t was remarkably long when compared to seasonal pond, which indicated more activity in the bottom water at seasonal pond. T_t was shorter in surface water at perennial pond, which indicated maximum heterotrophic activity. Surface water of seasonal pond also showed same trend except in monsoon season. The highest seasonal mean for the T_t of acetate was obtained, during monsoon in surface water of seasonal pond (Table 5) and the lowest mean was observed in pre-monsoon at surface water in perennial pond (Table 4). Spatial distribution of turnover time of acetate in Kiel Fjord was considerably less compared to Central Kiel Bight. Gocke (1977) found without exception turnover times measured at Central Kiel Bight are longer than at Kiel

Fjord. On the average, the ratio of T_t for acetate in Kiel bight and Kiel Fjord were found to be 5.6, which indicated longer turnover time at Kiel Bight. Whereas the vertical differences are considerably smaller. Turnover time of acetate and chlorophyll 'a' was found to be positively correlated (Table 33) no significant correlation existed between the bacterial variables and the chlorophyll-a concentration by Gocke & Rheinhimer (1991) when they conducted a survey of bacterial number, biomass and the activity along the mid-line of the Baltic sea. This lack of correlation seems surprising since several studies including the present investigation have shown significant correlation between chlorophyll concentrations and bacterial numbers (Ferguson and Rublee, 1976; Fuhrman et al. (1980) other researchers (Ducklow, 1984) however, were unable to detect such a correlation on a smaller spatial scale or on a relatively small span of chlorophyll concentration (Bird and Kalff, 1984). Correlation between chlorophyll concentrations and bacterial abundance and pigment concentration became obvious in fresh and marine waters (Ducklow and Kirchman, 1983; Bird and Kalff, 1984; Ducklow, 1984).

Recently developed techniques (^{3}H- Thymidine & ^{14}C-glucose) for estimating bacterial biomass and productivity indicated that bacterial biomass in the sea is related to phytoplankton concentration and that bacteria utilise 10 to 50% of carbon fixed by photosynthesis (Azam et al., 1983). Chandrika (1991) found that bacterial cell production rates varied between 0.07 and 0.25^{-1} based on empirical conversion factors. Carbon flux estimates are less in most of the observations which may be due

to high carbon demand for bacterial productivity, implying that a source other than the recent release of photosynthate may be necessary to balance the bacterial demand.

(b) Seasonal variation of maximum uptake velocity of Acetate (V_{max})

There was no similarity in the V_{max} of acetate in seasonal pond compared to perennial pond during pre-monsoon and post-monsoon season (Fig.11). The similarity in the values of V_{max} of acetate was noted, only in the monsoon season. The same type of observations was obtained with studies in the Kiel Fjord temperate waters (Gocke, 1977). It can be concluded therefore, that a (probably large) fraction of the bacterial population is able to take up the acetate, and the uptake was caused by distinct sub-populations which were present throughout the study period. However, Hoppe (1974) showed that almost all of the saprophytic bacteria of the study area were able to utilise the substrate acetate. The gross uptake velocity of acetate was always considerably higher when compared to glucose. This was especially very evident in perennial pond, where the heterotrophic activity were generally found high.

The average highest seasonal mean of V_{max} of acetate in perennial pond surface and bottom water was found during pre-monsoon, the values being 0.28 $\mu gC\ l^{-1}h^{-1}$ and 0.26 $\mu gC\ l^{-1}h^{-1}$ respectively while in seasonal pond, V_{max} of acetate was 0.27 $\mu gC\ l^{-1}h^{-1}$, during monsoon at surface water and 0.24 $\mu gC\ l^{-1}h^{-1}$ at bottom water during post-monsoon season (Table 4 & 5). In seasonal pond bottom water the differences in the V_{max} are less marked, whereas in surface water at perennial pond, the

V_{max} was found to be more in pre-monsoon season. Thus in both the ecosystem acetate is taken up about twice as fast as glucose. Investigations of other authors in eutrophic lakes also showed considerably higher uptake rates of acetate than of glucose (Allen, 1969; Francisco, 1970; Wright & Shaw, 1975; Ansback & Blackburn, 1980).

c) Seasonal variation of $K_t + S_n$ of acetate

In the present study seasonal mean of $K_t + S_n$ of acetate was the highest being 4.71 μgC l^{-1} in monsoon season in the bottom water of seasonal pond (Table 5) and the lowest mean 1.89 μgC l^{-1} was obtained during pre-monsoon at surface water in perennial pond (Table 4). The different behaviour of $K_t + S_n$ of acetate might be due to the fact that this substrate is not taken up by a transport enzyme which is specific to acetate. Variations in $K_t + S_n$ can be caused by changes in either the transport 'constant' or the natural substrate concentration or by simultaneous variations of both the parameters. Since high concentrations of dissolved organic componds certainly induce higher acetate uptake rates the observed parallel between the maximum uptake velocities and the $K_t + S_n$ values for acetate might be due to higher concentration of this compound in the aquaculture pond of Narakkal. However, another possibility of high peaks of V_{max} may be due to distinct bacterial group with high transport 'constant' dominating the uptake of acetate. Ansback and Blackburn (1980) found out the rate constant for acetate turnover at 4 to 6 cm depth did not vary greatly with season in the anoxic inshore

marine sediments of LIMFJORDEN, Denmark. They also found acetate is an obligatory product of fermentation and for this reason it was selected as an indicator of anoxic carbon flow in recent marine sediments. ($K_t + S_n$) of acetate and pH was also found to be positively correlated in surface water of perennial pond which might have enhanced the fermentation of acetate (Table 33).

The value of ($K_t + S_n$) of acetate in stratified reservoir in North Carolina, USA at 0.5 m and 4.0 metres (Francisco, 1970) indicated changing population and/or changing concentration of the same population. Large increases in the turnover time were associated with lower values of V_{max} and higher values for ($K_t + S_n$) this may be due to mixing of the bottom water with high acetate concentration and also due to bacteria which lack acetate transport system in the surface waters. Acetate was found to be concentrated in bottom waters due to its production through fermentation (anaerobic) pathways. The extreme variabilities of the variables of kinetic parameters ($K_t + S_n$) makes definite conclusions impossible. T_t of acetate and ($K_t + S_n$) of acetate was found to be positively correlated in perennial pond surface water. Chandramohan and Ramaiah (1987) found that the ($K_t + S_n$) values were very high in lagoon waters of Kavaratti Island. Oceanic water exhibited high T_t for glucose and acetate when compared to lagoon waters. Sodium acetate showed a maximum T_t of 507.08 in oceanic waters which is nearly 16 times higher than that in lagoon waters. ($K_t + S_n$) of acetate and V_{max} of acetate in perennial pond surface water was found to be positively correlated which indicated

the contribution of bacteria in the maintenance of substrate availability and utilisation. The $(K_t + S_n)$ of acetate tends to remain more constant during post-monsoon season in surface water in perennial pond (Fig. 13). The activity of bacteria was generally affected by monsoons and tides when the organic and inorganic matter were brought down and is of considerable importance in the maintenance of microbial life in the perennial as well as seasonal pond.

The parameter $(K_t + S_n)$ gives some idea of the natural substrate concentration. Small changes of $(K_t + S_n)$ suggests the presence of a mechanism which stabilises and controls the variations of the concentrations of dissolved organic substrate on a low level, (Andrew and Williams, 1971) at this low level a balance between release and uptake of organic substances is achieved which can be disturbed only for short period of time. This controlling function of bacteria towards maintenance of environment clearly can be attributed to the heterotrophic activity of microorganisms. The monsoon was characterised by general decline in heterotrophic activity and post-monsoon season was found to be productive over the two-year study period, when fertiliser input can be reduced considerably.

(b) Hydrological Related Factors

A change in temperature will disturb the existing steady state of heterotrophic activity of bacteria in the pond and in most cases the change will lead to the establishment of a new study state of activity. Thus, the effect of temperature can be expressed as a change of population

density and a change in enzyme kinetics. The average potential glucose uptake at maximum temperature (35°C) was (1.072 µgC $l^{-1}h^{-1}$) in the perennial pond and (35°C) (0.4395 µgC $l^{-1}h^{-1}$) in the seasonal pond surface water. Highest seasonal mean for temperature was recorded during pre-monsoon season in the surface water of the seasonal pond. In the sediment sample also the highest temperature was recorded only in the pre-monsoon that too in seasonal pond. The glucose uptake of microorganisms is limited by temperature. The sensitivity to temperature of microorganisms was high in the mixed water region and low in the stable region (Takahashi and Ichimura, 1971). In the present observation the temperature was low in stable perennial pond. The maximum heterotrophic uptake of glucose was also recorded in pre-monsoon during 1989 (Fig.6) in perennial pond. The V_{max} was found to be less and somewhat constant in monsoon and post-monsoon in bottom water of seasonal pond during 1990. The glucose uptake at the temperature measured in the present study was considered to be proportional to the biomass of bacteria in the pond, considering the profile of potential glucose uptake (Fig.5) the heterotrophic activity were abundant not only in shallow seasonal pond but also found to be high in perennial pond. The maximum activity from the uptake of C^{-14} labelled glucose in bottom water was 1.361 µgC $l^{-1}h^{-1}$ in February 1989 (Fig.6) and the uptake reflects the abundance and biomass of saprophytic bacteria in seasonal pond. Sorokin (1970) also measured maximum bacterial activity in deeper waters of tropical pacific ocean.

Hobbie and Wright (1966) reported that the in situ glucose uptake was 0.1 to 10 µg glucose/l/hr at 1°C to 10°C in eutrophic lakes and 0.01

to 0.1 µg glucose/l/hr at the same temperature range in oligotrophic lakes. Allen (1969) also observed seasonal changes in the glucose uptake in the range of 0.12 to 15 µg glucose/l/hr in a shallow eutrophic pond, in the northern pacific ocean the uptake at in situ temperature was 0.024 and 0.003 µg glucose/l/hr in the layers above and below 100 meters respectively.

The heterotrophic activity of acetate measured by the kinetic approach and described by Wright and Hobbie (1966), and Griffiths (1982), indicated maximum velocities of the potential substrate uptake (V_{max}) during spring and summer. In the present study a very good negative correlation between turnover time of acetate and temperature was noted in bottom waters of perennial pond (Fig.9) during pre-monsoon months (March 1989). T_t 18.626 hrs and maximum velocity of uptake 0.3282 µgC $l^{-1}h^{-1}$ was also found to be maximum which indicated the possibility of floating population to be more. When T_t increased bacteria attached tc organic matter are likely to be more in the sample. Hanson and Wiebe (1977) suggested that 80% of the uptake was by particles less than 3 µm diameter and 93% was by particles less than 8 µm. Hence they showed that the bacteria not attached to particles comprise only 80% of the activity. Almost all the uptake by this fraction was found to be carried out by bacteria attached to detritus.

A sudden rise in temperature will kill off some microbes and cause other to form spores and leave the third group unaffected - an obvious way of succession of activity in aquaculture pond. All enzyme activity will be inhibited by the bactericidal action of high temperature and the

lipid composition of almost all microorganisms are altered with temperature. As temperature decreases, the relative content of the unsaturated fatty acids in the cellular lipids increases. This is an adaptation to temperature in microorganisms. Bell et al. (1980) found heterotrophic activity is influenced by temperature in two Cannadian rivers and Wood (1940) found that 42% were killed at 37°C and 15% survived at 45°C in warm currents of eastern Australia. In the present observation the maximum temperature was observed in pre-monsoon months and V_{max} of acetate had a positive correlation with temperature (Table 33). Liston (1957) found that bacteria from Aberdeen bay grew best between 0°C - 20°C and nearly all were killed at 37°C. He considered that the differences in temperature tolerance that have been observed in different localities and due to selection by environment.

Salinity itself does not affect bacterial activity but merely regulates the strains of bacteria responsible for the various microbial processes in the pond. The maximum salinity was observed during pre-monsoon months (Fig. 17) attaining 23.78‰ in 1989 and 24.92‰ in 1990; during March (Fig.18) at perennial pond and in seasonal pond, also the maximum value was recorded during pre-monsoon season (23.39‰) in 1989 and 25.9‰ in 1990 during April). Salinity was highly unstable throughout the study period due to tidal influence and monsoonal effect. A strong positive correlation was obtained for salinity with total heterotrophs but none of the kinetic parameters were found to be influenced by salinity in the present study. The salinity variation has been shown to affect the maximum

growth temperature of marine bacteria (Stanley and Morita, 1967) and to affect the ability of marine microbes to synthesise protein (Copper and Morita, 1972). The effects of ionic menstrum on marine bacterial physiology has been studied by MacLeod et al. (1958) Payne (1960); Stevenson (1966); O'Brien and Stern (1969 a,b) and Wong et al. (1969). The effect of salinity on metabolic pathway of obligatory psychrophilic marine bacterium Vibrio marinus was studied by Griffiths and Morita (1973) and found that the patterns of CO_2 release over most of the range tested showed that the hexose monophosphate (HMT) pathway is greatly affected by the changes in salinity and the changes observed with salinity are not ion specific for Na or Cl. The environmental salinity have got important implications in the release of fragments of glucose skeleton back into the environment. It is very evident from the figure 5 that the highest salinity affected the glucose uptake level from specific labelled C^{14} glucose during 1989 and the hexose monophosphate (HMT) pathway is greatly affected by the changes in salinity (Griffiths and Morita, 1973). The enhanced salinity in pre-monsoon months during 1990 was found to have no effect on V_{max} of glucose in both the ponds. The salinity relationship between seasons was found to be significant. The highest V_{max} was encountered when highest salinity was prevalent in both the ponds indicated the stability of marine bacterial enzymes at higher salinity levels in the aquaculture pond. $K_t + S_n$ values closely followed changes in V_{max} values. This was proved by Valdes and Albright (1981) who has found that the V_{max} and $K_t + S_n$ ratio's of Frazer river (0‰ S) to Strait

of Georgia 25‰ S were 2.3 and 2.7:1 respectively with lower ratio noted at intermediate salinity. The lowest ratio's were noted 19% S water. The turnover time of glucose exhibited a continuous decrease at 19% S water and then increased in 25‰ S. Salinity decrease had not affected glucose heterotrophic uptake activities, although the activity decreased considerably. The heterotrophic potential (V_{max}), as total activity, decreased 45% with reduced salinity whereas the $K_t + S_n$ values increased by 30% from 23‰ to 2‰ salinity S water (Valdes and Albright, 1981). All the kinetic parameter were influenced more by temperature rather than salinity probably because the salinity changes are not sufficient to alter them.

The highest dissolved oxygen values were found during monsoon months (July and August) 5.88 mg/l and 6.86 mg/l in 1989 and during 1990 also it was found to be high in monsoon month (6.768 mg/l in July) at perennial pond, whereas in seasonal pond the high values were obtained only during pre-monsoon months (April and May - Fig.17). The highest oxygen values indicated low heterotrophic activity. The low oxygen values indicated the oxygen demand of organisms also in the sediment eventhough the dissolved oxygen from the surface penetrates only on a limited distance depending upon the degree of turbulance at the sediment water interface (Sepers et al., 1982).

Low pH was encountered during the post-monsoon season in both the years the value being 6.56 in November 1989, 6.5 in July 1990 at

perennial pond, and 6.3 in June at seasonal pond. Usually the pH changes were used to calculate carbondioxide variation and to derive a figure for the productivity of the area. Weichart (1980) recently published a study on diel pH changes from which he calculated changes in carbondioxide. Ferroni et al. (1983) reported that turnover time for glucose increased with decreasing pH and V_{max} values also decreased with decreasing pH. In the present study $K_t + S_n$ of acetate in the perennial pond was found to be positively correlated with pH (Table 33).

(c) Phytoplankton - related factors

Primary productivity related factors

Net primary production was always lower than the gross primary production in perennial pond during 1989 and 1990, but in seasonal pond net production was somewhat higher than gross production in the month of October 1990, which may be due to microbial metabolism. Hobbie and Rublee (1977) demonstrated a close relationship between phytoplankton primary production and the potential bacterial uptake of organic substrates. Several workers have shown that EOC released from phytoplankton can be utilised by bacteria (Bell and Mitchel, 1972; Herbland and Pages, 1975; Nalewajko et al., 1976; Iturriaga, 1981). The extra cellular organic carbon (EOC) released by phytoplankton population is of importance to bacterial heterotrophy and thus to the carbon and energy flow in the aquatic environment (Larson and Hagstrom, 1979).

The overall concentration of organic solutes in the aquaculture pond is constant, because of close coupling between the unstable portion

of organic material dissolved in seawater. The heterotrophic population has indicated by correlations between primary production and heterotrophic activity. Once phytoplankton have fixed carbon in phytosynthesis some may be lost as CO_2 in respiration and some as organic matter in excretion. The oxygen Light-Dark productivity method provides gross and net productivity whereas C^{14}-method is usually considered to give something between net and gross primary productivity with 24 hrs incubation. The C^{14}-method gives an estimate of net primary productivity (Eppley and Sharp, 1975). If respiratory losses are large and variable, short term incubations may not give a good assessment of real productivity. Indeed the large and variable respiratory losses are due to phytoplankton or by the full microbial community. Hence one should be very cautions in interpreting the results of short term incubations. Long incubations are subjected to potential error from bacterial respiration, zooplankton excretion and physiological stress.

(d) Bacterial distribution

Total aerobic heterotrophic bacterial distribution

The density of total heterotrophs had strong positive correlation with salinity in the perennial pond as well as seasonal pond (Table 33 & 34) and no other environmental variables had positive correlation with total number of heterotrophs; whereas a negative correlation was obtained with dissolved oxygen in surface water of perennial pond (Table 33) whereas Jana et al. (1980) found the population size of water bacteria was inversely

correlated with the variation of temperature and dissolved oxygen of water. Bent and Goulder (1981) found significant correlation between density of attached bacteria and temperature and salinity and concentration of suspended solids. It is very clear from the figure 27, total heterotrophs predominated in the post-monsoon season in both the year. Although there was general tendency of higher number of populations in the sediments, of all the samples, 80% of the water sample contained more population than the sediment in the present observations. Comparison of the maximum number of the bacterial population per ml of water and per gram of sediment for each month indicated that highest count 216 x 10^6/gm in January 1989 in sediment and 256 x 10^5/ml in water during March 1989 at perennial pond. Gocke and Rheinhimer (1988) found no significant correlation between temperature and total bacterial numbers. In the present observation also no statistical relationship was found between temperature and total bacterial count. Increases in total number of bacteria (Fig. 27 & 28) were generally related to increases in V_{max} for both glucose and acetate in surface and bottom water. The assumption of previous investigators (Allen, 1969; Hobbie, 1967; Wright and Hobbie, 1966) as to the relation of V_{max} to bacterial numbers thus seem to be correlated in the present ivestigation. Russian workers have also found a direct relation between chemo-organotrophy and bacterial numbers. Since the increases in V_{max} are not directly proportional by a constant factor to the total bacterial concentration it can be assumed that the population changed both quantitatively and qualitatively. Although the concentrations of bacteria found in the present study are higher than

normally found in fresh water, they are comparable to results recently obtained with similar techniques (Kuzhetsov, 1968; Straskraba and Straskrabova, 1969).

Any relationship of V_{max} for glucose and acetate to the concentration of viable bacteria (Fig. 27 & 28) was distinct. The relationship was particularly clear in the bottom water which was not surprising since the microorganisms from this anaerobic and microaerophilic environment were incubated aerobically. The relationship of viable bacteria to V_{max} was an expected one, since growth conditions were quite different in situ culture experiments. But Hobbie, Hamilton and Allen (1970) have observed this non-relationship and ascribed it to the selective enrichment of culture.

Using the technique of Parson and Strickland (1962), which was further improved by Wright and Hobbie (1966), several studies of heterotrophic activity have been performed in marine and fresh water environments. To give an impression of the great diversity of heterotrophic activity in different areas in Indian waters some data are compiled in Table 35 (C^{14}-glucose) and Table 36 (C^{-14}-acetate). These data are only momentary pictures not reflecting seasonal variations. These data clearly illustrates the great variability of heterotrophic activity in different marine regions. Table 35, shows the results of Gocke et al. (1981) the range in concentration between the totally different biotopes is quite low. The values reflected in no way the degree of eutrophication. Inner Kiel Fjord showed highest T_t, 45.3 hrs in spring whereas in Lakshadweep the values more than 175-44 hrs was recorded by Chandramohan and Ramaiah (1987)

Table:35. Heterotrophic Activity obtained by tracer method (^{14}C-glucose) of various areas.

Seasons	Locations	Substrate	V_{max} ($\mu gC\ l^{-1}h^{-1}$)	T_t (hrs)	$K_t + S_n$ ($\mu gC\ l^{-1}$)	Reference
Winter	Inner Kiel Fjord	Glucose	0.041	45.3	1.92	
	Schreventeich	"	0.357	2.93	1.04	
Spring	Inner Kiel Fjord	"	0.136	6.71	0.92	Gocke et al.(1981)
	Kiel Bight	"	0.115	5.39	0.62	
	Stocksee	"	0.103	10.8	1.11	
	Schwentine	"	0.532	5.12	2.72	
	KAVARATTI					
	Lagoon Water	Glucose	1.165	20.94	21.62	
April - 1984 to January - 1985	Oceanic Water	Glucose	1.41	31.44	41.09	Chandramohan,D. & Ramaiah.N (1987)
	AGATTI					
	Lagoon Water	"	0.279	175.44	38.42	
	COCHIN - NARAKKAL					
*Pre-Monsoon	Perennial Pond S.Water	"	0.447	9.68	3.475	
	Seasonal Pond S.Water	"	0.022	10.12	1.744	
*Monsoon	Perennial Pond S.Water	"	0.384	9.48	2.928	Present study(1992)
	Seasonal Pond S.Water	"	0.191	11.76	2.068	
*Post-Monsoon	Perennial Pond S.Water	"	0.307	11.13	2.8	
	Seasonal Pond S.Water	Glucose	0.18	8.44	1.03	

* Present observation, S.Water - Surface water

that too in Agatti lagoon water. The values of T_t found in the present investigation was resumbling more of the Gocke's data collected in the inner part of the Kiel Fjord of the Northern Germany especially the Stocksee samples. The maximum T_t for glucose was obtained in seasonal pond surface water sample in the present study. The T_t ranged from 8.44 to 11.76 hrs for different seasons. V_{max} of glucose was found to be maximum in Gocke's observation in a small river influenced by sewage discharges during spring. The lowest was recorded in the inner Kiel Fjord (Table 35). Chandramohan and Ramaiah recorded highest V_{max} (1.165 μgC $l^{-1}h^{-1}$) in the lagoon water of Kavaratti island. In the present study lowest V_{max} (0.18 μgC $l^{-1}h^{-1}$) was observed in seasonal pond surface water in post-monsoon season and the highest in the perennial pond surface water samples (0.447 μgC $l^{-1}h^{-1}$) in the pre-monsoon season. Of all the observation (K_t + S_n) value recorded in the oceanic water of Kavaratti island was predominant (Chandramohan and Ramaiah, 1987). The marked variations in all these findings can be interpreted as a result of heterogeneity of the bacterial populations.

Table 36 illustrates tht the turnover time of acetate is more in oceanic water when compared to lagoon water. Maximum T_t was obtained during monsoon period in seasonal pond surface water in the present study. The maximum was obtained in perennial pond surface water during pre-monsoon season. V_{max} of acetate was very less in oceanic water of Kavaratti island. Maximum V_{max} was obtained lagoon water of Kavaratti island. Maximum V_{max} of acetate was somewhat constant in all the

Total 36. Heterotrophic activity obtained by tracer method (^{14}C-Acetate) at various areas.

Seasons	Locations	Substrate	V_{max} ($\mu gC\ l^{-1}\ h^{-1}$)	T_t (hrs)	$K_t + S_n$ ($\mu gC\ l^{-1}$)	Reference
	KAVARATTI					
April - 1984 to January - 1985	Lagoon Water	Sodium Acetate	1.14	31.44	41.09	Chandramohan, D. & Ramaiah, N. (1987)
	Oceanic Water	Sodium Acetate	0.035	507.08	16.585	
	COCHIN - NARAKKAL					
*Pre-monsoon	Perennial Pond S. Water	"	0.277	6.95	1.86	
	Seasonal Pond S. water	"	0.211	11.71	2.16	
*Monsoon	Perennial Pond S. Water	"	0.242	10.44	2.932	
	Seasonal Pond S. water	"	0.267	17.31	3.9	Present Study (1992)
*Post-monsoon	Perennial Pond S. Water	"	0.249	7.09	1.89	
	Seasonal Pond S. Water	Sodium Acetate	0.237	10.70	2.29	

* Present Observation, S. Water - Surface Water

seasons in the present study the range between 0.211 μgC $l^{-1}h^{-1}$ in seasonal pond surface water to 0.277 μgC $l^{-1}h^{-1}$ in perennial pond surface water in pre-monsoon months ($K_t + S_n$) values was found to be very high in lagoon water of Kavaratti island. The $K_t + S_n$ value ranged from 1.86 μgC l^{-1} in perennial pond surface water in pre-monsoon months to 3.9 μgC l^{-1} in seasonal pond surface water during monsoon.

Conclusion

T_t of glucose is relatively high (18.325 hrs) in bottom water, whereas T_t of acetate was somewhat constant from August 1989 to April 1990 (8.095-15.74 hrs) in bottom water in perennial pond. While T_t remained relatively constant in bottom water, V_{max} and $K_t + S_n$ was also found constant from September 1989 to June 1990 in both the kinetic parameters. There are no statistical relationships among environmental variables and the observed kinetic parameters. In bottom water $K_t + S_n$ and total bacterial number had inverse relationship which may be due to the monsoon effect. While V_{max} of glucose usually increased prior to monsoon and continues at high level during and just after it. This may be due to the chemo-organotrophs utilising the substrate and preventing the accumulation of glucose in the surface water in perennial pond. V_{max} of glucose appears to be directly proportional to total number of aerobic heterotrophs in pre-monsoon months, except in monsoon. This indicated that a qualitatively and quantitatively changing bacterial heterotrophic community is responsible for the velocity of uptake of glucose and other substrates in the aquaculture pond. The concentration of viable heterotrophic bacteria is related to salinity and oxygen determined in this investigation.

T_t of acetate and chlorophyll-a, V_{max} of acetate and temperature, $K_t + S_n$ of acetate and pH was found to be positively correlated. Also T_t of acetate and $K_t + S_n$ of acetate and V_{max} of acetate was found to have direct relationship with each kinetic parameter. All the acetate uptake results had strong significant, statistical validity than for glucose. This may be due to experimental error caused by the very high rates of uptake of acetate, during the experimental period. Acetate removal from the anaerobic bottom water samples followed diffusion kinetics rather than Michaelis-Menten. V_{max}, $(K_t + S_n)$, total heterotrophs and other environmental variables were highest in the pre-monsoon season. Extreme instability was osberved throughout the period of study in the extend of chemo-organotrophic utilisation of glucose and acetate. In monsoon values of V_{max}, $(K_t + S_n)$, and total heterotrophs was generally low. While values of T_t of acetate was found to be high (3.07-15.58 hrs).

In seasonal pond there was a good correlation between T_t of glucose in surface water and total heterotrophs in pre-monsoon season V_{max} and total heterotrophs was found to be very high prior to monsoon just like perennial pond. If the estimates based on uptake of labelled substrates, were correct, it shows between 40 to 90% of free living bacteria were active and that 80 to 90% of heterotrophic activity was due to smallest size fraction of aerobic heterotrophs, whereas the attached bacteria are important in degradation of larger organic particles. There was inverse relationship between $(K_t + S_n)$ and total bacteria which may be due to other environmental variables especially temperature. Heterotrophic uptake of acetate was found to be less in the monsoon period, especially in the

month of August, which may be due to sudden decline in temperature. The results of this investigation shows that the chemo-organotrophic community responded very rapidly to changes in temperature, substrate concentrations and oxygen concentrations by altering the total aerobic heterotrophic quantitatively and qualitatively. The source of glucose and acetate in the perennial and seasonal aquaculture ponds may be through phytoplankton excretion and/or dissolution of phytoplankton after death. It is evident from the rate of their utilisation, that they are very important substrates for the chemo-organotrophic aerobic heterotrophic community for obtaining energy both in the perennial and seasonal ponds.

V. SUMMARY

The study on "Heterotrophic bacterial activity in selected aquaculture systems near Cochin" was carried out based on the data collected in perennial and seasonal pond over a two year period from January 1989 to December 1990 to know the seasonal and vertical pattern of bacterial chemo-organotrophy by measuring glucose and acetate utilisation. ^{14}C glucose and acetate were used as tracers to study the uptake and incorporation of glucose and acetate by natural bacterial populations in the aquaculture ponds. The technique was useful in measuring glucose and acetate incorporation rates as low as 0.5 n ML^{-1}. The incorporation followed saturation kinetics. The relationship of environmental variables to bacterial chemo-organotrophy was also found out to understand the factors controlling microbial processes and the total energy flow in this ecosystem. The findings are summarised under four headings: Heterotrophy relationships, hydrological related factors, primary productivity related factors and relationship with total bacterial parameter. An account of heterotrophic activity in the aquaculture pond along with their relationships with environmental parameters are given with intensity charts and tables.

2. The heterotrophic activity was generally affected mainly by monsoons and tides when the organic and inorganic matter were brought down to aquaculture pond and is of considerable importance in the maintenance of microbial life in the perennial as well as seasonal pond.

3. The monsoon was characterised by general decline in heterotrophic activity and post-monsoon season was found to be productive over the

two year study-period, when fertilizer input can be reduced considerably.

4. The substrate turnover time (T_t) varied seasonally; substrate concentration S_n ($K_t + S_n$) varied little; V_{max} was directly proportional to the concentration of transporting enzyme (i.e. heterotrophs).

5. V_{max} of glucose had negative correlation with T_t of glucose which indicated that whenever velocity of glucose uptake increased turnover time decreased. Also $K_t + S_n$ of glucose and V_{max} of glucose had positive correlation which distinctly showed that the increase in substrate availability, velocity of uptake increased without inhibition.

6. V_{max} of acetate had direct positive correlation with $K_t + S_n$ of acetate and temperature for perennial pond. T_t of acetate had positive correlation with $K_t + S_n$ of acetate and chlorophyll-'a' which indicated the source of acetate, for these bacteria is from dead phytoplankton and the substrate availability controlled turnover time of the substrate.

7. In perennial pond, surface water ($K_t + S_n$) had direct positive correlation with pH.

8. The values of V_{max} tended to increase with total aerobic heterotroph but the high variability of number and V_{max} values in different seasons suggested quantitative and qualitative changes in the bacterial population.

9. In seasonal pond, T_t of acetate and dissolved oxygen in surface water and $K_t + S_n$ of acetate in bottom water was found to be significant over seasons.

10. The concentration of total aerobic heterotrophs (plate counts by pour plate techniques) was not related to heterotrophic uptake parameters and found negatively correlated with dissolved oxygen in both the ponds.

11. Eventhough salinity was found to be significant over seasons in surface water of both the ponds total heterotrophs showed positive correlation with salinity.

12. V_{max} increased with depth for both substrates during the premonsoon period. $K_t + S_n$ values varied not only from substrate to substrate but also with (V_{max}) in seasonal pond.

13. The activity parameters were somewhat more variable than bacterial numbers and biomass.

14. Acetate activity was more when compared to glucose activity.

15. The kinetic parameters of glucose and acetate utilisation did not vary considerably between surface water and bottom water.

16. T_t, V_{max} and ($K_t + S_n$) increased for both the substrates in premonsoon months. This probably reflected the general increase in activity of the entire biological community during the period and also can be attributed to higher temperature.

17. In seasonal pond T_t of acetate and dissolved oxygen in surface water, $K_t + S_n$ of acetate in bottom water was found to be significant over seasons. Also, T_t of acetate was found to be positively correlated with chlorophyll 'a' indicating direct dependence of bacteria on DOM coming from phytoplankton.

18. Kinetic parameter (V_{max} of acetate) and total heterotrophy appear to be restricted in their distribution by temperature rather than salinity probably because the salinity changes are not sufficient to alter them.

19. T_t of acetate and $K_t + S_n$ of acetate was also found to be significant which indicated that turnover time of acetate and substrate concentrations of acetate vary considerably in surface and bottom water.

20. The total heterotrophs had strong positive correlation with salinity which indicated that just like perennial pond, seasonal pond also harboured more halophilic bacteria than non-halophilic forms requiring Na^+ and Cl^- ions. Total plate count and salinity in perennial pond was found to be significant over seasons.

Oxygen levels have negative correlation with total heterotrophs showing that these two ponds were predominated with more number of anaerobic, micro-aerophilic forms than heterotrophic aerobes.

21. Total number of heterotrophs obtained by plate counting techniques was less than expected but the data showed distinct seasonal succession of bacterial types and that there was consistant differences between seasonal and perennial ponds. Psychrophilic bacteria dominated during monsoon and psychrophilic and mesophilic bacteria dominated in post-monsoon season. All these results demonstrate that the microbiological parameters in both the ecosystem are controlled more by a concentration gradient.

22. The chemo-organotrophic microbial community responded very rapidly to changes in temperature, substrate concentration, and oxygen concen-

tration by altering the bacterial population quantitatively and qualitatively. Glucose and acetate are produced through phytoplankton excretion or dissolution of phytoplankton after death and because of high rate of glucose and acetate utilisation it is to be concluded that they are very important substrates for the maintenance of fertility of the pond by chemo-organo-trophic community.

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